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Editorial Views and News

In this issue, the Proceedings of the last annual I.A.D.R. meeting, held in March of 1961, are duly published. This fact leads one to think of the next meeting, which is scheduled for St. Louis in 1962. It will be held on March 15–18 in the Hotel Jefferson, followed by the annual meeting of the American Association of Dental Schools. More information on the meeting will be forthcoming when available, but it is apparent that the deadline for submission of abstracts for the meeting must be no later than December 1, 1961. As indicated in the Proceedings of the last meeting, funds were made available for the publishing of a second biographical directory of all Association members in good standing. Forms have been sent to all concerned, and the data are being received in considerable quantities in every mail delivery here at the *Journal* editorial office. The space devoted to "Views and News" regarding the Association and the *Journal* is open to individuals connected with the Association and concerned with its welfare. Richard S. Manly, Associate Editor and long an active I.A.D.R. member, has submitted the following interesting editorial report. He is in the Department of Research at Tufts University School of Dental Medicine in Boston.

Distribution of Research Contributions Past and Future Meetings.—During the last four years the number of papers presented at the annual I.A.D.R. meetings has increased from 248 to 356, and the contributing institutions from 69 to 99; yet the average number of papers per institution has remained surprisingly constant during the growth (Table 1), even though the number of papers presented ranged from 1 to 25 per institution. It seemed worthwhile to make some statistical examination of the distribution in order to learn whether the data obey "normal" laws. This has been done and the data are plotted in Figure 1. On the ordinate is plotted the percentage of the institutions presenting the particular number of papers indicated on the abscissa. (The abscissa is on a logarithmic scale, and the ordinate is on the probability scale.) For example, in 1958 and 1959, 5 per cent of the institutions presented 2 or more papers at the national meeting, which is represented by the uppermost point on the chart. The lowermost point on the curve is the one institution in 1960 that presented 25 papers.

TABLE 1

Year	1958	1959	1960	1961	1970
Site of meeting	Detroit	San Francisco	Chicago	Boston	
Total papers	248	285	301	356	665
Total institutions	69	83	84	99	189
Average number of papers per institution	3.6	3.4	3.5	3.6	3.5
Normalized distribution; institutions presenting					
5 or more	16	20	20	24	45
10 or more	7	8	8	9	19
15 or more	3	4	4	5	9
20 or more	2	2	2	3	6
Leading contributing institutions	Indiana N.I.D.R. Eastman Tufts U. Illinois	Eastman Indiana Tufts U. Alabama Harvard	Indiana Harvard N I.D.R. Eastman Loyola U. Illinois U. Pennsylvania	Indiana Harvard Tufts N.I.D.R. U. Alabama	

The data for all four years correspond well to a straight line, indicating that the distribution of the number of papers obeys probability laws. If 58 per cent of the organizations present two or more papers, then 1 or 2 per cent of the institutions will present 25 papers. The distribution curve is relatively constant from year to year and is uninfluenced by the site of the meeting, even though two meetings were in the middle of the country (1958 and 1960) and two meetings were

in the east and west extremes. Among institutions presenting 10 or more papers, the 1961 Boston meeting seems to have been preferred by a slight margin.

The 5 institutions that presented the most papers are also given in Table 1. Certain names reappear frequently. These are Indiana University, National Institute of Dental Research, Harvard University (including Forsyth), Tufts University, Eastman Dental Dispensary, and the University of Alabama. Apparently there is a reasonably stable population of high contributors.

This stability and the uniformity in distribution suggests the possibility of making reasonable predictions of the distribution of papers at a national meeting in 1970. From 1958 to 1961 the total number of institutions presenting papers increased on the average of 10 per year, which leads to a figure of 189 institutions presenting papers in 1970. There appears to be no logical limit to the number of new institutions that begin to do dental research, now that there are twice as many institutions presenting papers as there are dental schools. It seems logical to assume the continued growth of 10 institutions per year. On this basis we would expect a total of 665 papers, or nearly twice the number of papers we had in 1961. The number of institutions presenting 5, 10, 15, or 20 or more papers will likewise be doubled.

These calculations would support the need for formation of special groups of the I.A.D.R., each with its own program, perhaps for lifting the restriction on the number of papers presented orally by one person, and for showing geographical imagination in the location for future meeting sites. Future meetings are already being planned for more distant sites rather than the continental midwest of North America, where most of the past meetings were held.

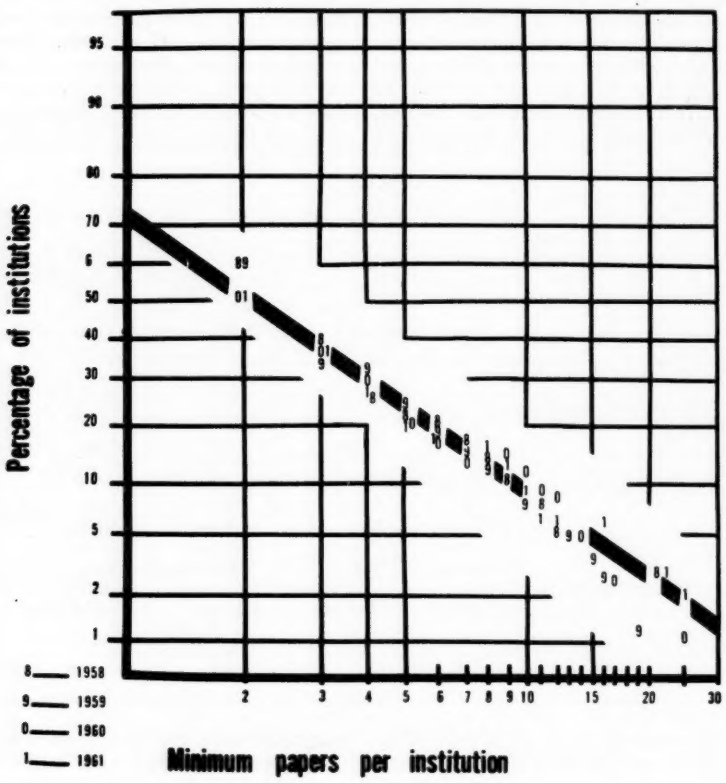


FIG. 1

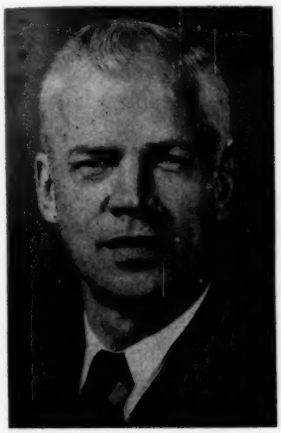
The author wishes to acknowledge the assistance of Miss Viola Fucci in compiling and calculating these data.

—F. J. O.

*Presidential Inaugural Address**

International Aspects of Dental Research

JAMES A. ENGLISH
University of Buffalo, New York



I have selected as my subject tonight "The International Aspects of Dental Research." I believe this to be an important subject, and I have solicited comments from a number of people on how they believe our Association is serving an international purpose, whether they believe it is possible for us to become a better international organization, and how this might be accomplished.

I suspect that the majority of the persons in this banquet hall have given little thought to the internationality of our Association, that relatively few recognize that there are any serious problems related to international membership, and that there will be a number of our most active members who will be in varying degrees of disagreement with the suggestions and conclusion I shall present this evening. I do not intend to do much more than give an outline of my inquiries into the question of internationality for I realize that a long discourse would be out of place following the social activities we have had so far this evening, but I do hope that what I say may lead to some action in subsequent council meetings. Most of all, I hope you will be interested in what I have to say.

I believe a good way of introducing my discussion is to read a letter I received four weeks ago from a friend in Bristol, England:

Dear Jim: Many thanks for your letter. I think it is fair to say that there is a good deal of feeling in this country, that so far as the individual research worker is concerned, to join the IADR means paying three dollars per annum and getting little or nothing in return. It is true that you publish our abstracts, but we could get them done here.

We feel there is some value in an international association, but even I find it difficult to justify this to my colleagues. As you may know, we have about 80–100 people attending the meetings of the British Division, of which only seventy or so admit that membership in the IADR is valuable, by joining.

Again, we now have a journal in this country (Archives of Oral Biology) which is a good journal, and I am sure they would be very pleased to publish our proceedings. Some have, indeed, suggested that we should ask them to do so.

* Presented by the incoming president on the occasion of the annual banquet of the International Association for Dental Research, Saturday evening, March 25, 1961.

At one time, it would be argued that membership in the IADR indicated at least some contribution to dental research. We have the feeling that, with the easing of the requirements for membership, this is no longer true. Again, as you suggest, it seems ridiculous to state that this is an international association when it has never been known to have meetings outside the U.S.A. It may be true that the membership is much greater in the U.S.A., but I do not think we should speak of the total membership, but rather of percentage membership in relation to populations.

So far, I have been stating views expressed to me and to several colleagues. I, myself, think that if the IADR hopes to persist as an international organization, it must make some effort to move the annual general meeting around the world, and give at least an opportunity for a non-American to hold office. It might also encourage local international groups, e.g., European, to meet biannually or so and I think that every four or five years, the annual general meeting should come to Europe.

As you will appreciate, these are private views and are not the opinion of the British Division. They are expressions of opinions which have been given to me and my two collaborators, but we know that there is a fair number of people who think in this way.

Sorry, but you asked for both barrels. Yours sincerely. . . .

Now this letter from England does indeed express a common opinion, for I have replies of this kind from a dozen people living in several different countries. Perhaps now we should examine the structure of the IADR as it was originally conceived.

It has been just over forty years since our Association was founded in New York City by twenty-one scientists who were interested in dental research. A lot can happen in forty years, and, if you doubt this, let me remind you of the development of one branch of science in that time. About forty years ago, I was living in southern Pennsylvania, and I can still remember the day when our teacher let us go home at noon because it was stated in the morning newspaper that there was to be an airplane passing over our town that afternoon and she wanted us all to have the opportunity to see it. The developments in dental research since 1921 may not have been as dramatic as those in aeronautical engineering, yet all of us could point out many new concepts that are valid today. Surely, it is time that we re-evaluate the objectives of the founding fathers of IADR to see if they are equally valid today.

The original constitution of this Association created an international body composed of national divisions and local sections. It was the specific intention of the organizers of the Association that divisions would be established in several nations, the first division being the American Division. The first three sections were established in New York, Boston, and Chicago in 1921, and, within three years, there were additional sections organized in Toronto and San Francisco.[1] Although the articles of agreement specified that it was the intention of the Association to facilitate co-operative effort and achievement among investigators in ***all nations*** in order that dentistry could render more service to humanity ***throughout*** the world, there was not a very specific delineation of the international mechanisms which would accomplish this objective.

When one considers the influence that investigators from Europe played on the early development of dentistry in this country, it is not surprising that an international association was conceived. The early foundations of the pathologic processes occurring in the mouth were formulated in France, Germany, and Austria, and the acidogenic

theory of dental caries of Miller was early accepted into American teaching. Several decades ago, numerous scientists in dental fields came to this country from Europe, particularly from Germany and Austria, and became well established in the teaching of more fundamental areas in schools of dentistry. This pattern of internationalism has continued through the years, and many of our dental schools have one or more members on their staff at the present time whose training, in part, was gained in Europe and other continents prior to emigrating to this country.

Today, there are four divisions of the International Association—the American Division, the British Division, the Scandinavian Division, and the Japanese Division. The American Division holds its meetings at the same time as the annual meeting of the International Association for Dental Research, and the British Division holds annual meetings, which, incidentally, have had substantial increases in numbers of papers during the last few years. In addition to the 80–100 attendees at the British meetings and 30 in the three countries which comprise the Scandinavian Division, there are 36 members of the Association scattered throughout Continental Europe, but nowhere are there sufficient numbers to form a division, except in Great Britain and the Scandinavian countries. There are 30 members of the Japanese Division. There is little doubt that the fighting of two world wars in the first half of the twentieth century has been one major factor in the restricted growth of internationalism in our Association. As you know, the Scandinavian countries have a research organization of their own called Nordiska Odontologiska Föreningens, which meets biennially in one of the larger cities of Scandinavia. Elsewhere on the Continent there are several research organizations directed toward specific areas of dental science as, for example, ARPA (Association pour les Recherches sur les Paradontopathies), which emphasizes periodontal research, and ORCA (European Organization for Research on Fluorine and Dental Caries Prevention), which emphasizes research on fluoridation. In Germany there are societies in which emphasis is placed on research in surgery or orthodontia. When one examines the increased emphasis that has been given to research in university dental schools throughout the world, it seems that it might be well to re-examine the organizational structure which was drafted into the International Association for Dental Research, to see whether the international pattern should be strengthened or whether, in truth, it is better to encourage individual nations or smaller groups of nations to organize research among themselves.

In this problem which I am presenting for your consideration, there are five main areas which I would like to discuss briefly with you: (1) Do we really want IADR to be international? (2) the general importance of international organizations and advantages of an international organization for dental research; (3) the sacrifices and difficulties related to internationalism; (4) finding financial support for international meetings; (5) suggested mechanisms for strengthening our foreign relationships in IADR.

I believe I can introduce the first topic—i.e., Do we really want IADR to be international?—by reading a portion of a letter from a friend in London, England:

". . . I rather doubt whether the majority of members of IADR want it to be international; the whole constitution is against any sort of international control of the organization. However, an increased international flavor could be produced by having occasional international meetings of the type which took place before the Centennial

Meeting of the ADA. These, it seems to me, should be held in different parts of the world as convenient, and perhaps about every three years. The present structure of IADR is built on the assumption that the central organization and the North American Division are synonymous, and this may not always be desirable. At present, non–North American members of IADR feel their connection with it to be very tenuous. If they pay their subscription, they feel they get nothing for it and have no voice in the affairs of the Association, as their representatives cannot attend the meetings of the Council or annual meeting except on rare occasions, and, of course, none of their subscription is available for the activities of their own division."

I believe we must realistically search our innermost feelings and decide whether we believe the advantages of an international organization outweigh the disadvantages. We must also face the fact that we are not truly international now if one defines the term in a modestly rigorous way.

The importance of strengthening our international bonds may be well introduced by quoting from a statement made by a group of world-renowned scientists at the Third Pugwash Conference held in Austria in 1958:[2]

"We believe that, as scientists, we have an important contribution to make toward establishing trust and cooperation among nations. Science is, by long tradition, an international undertaking. Scientists with different national allegiances easily find a common basis for understanding; they use the same concepts and the same methods; they work toward common intellectual goals, despite differences in philosophic, economic, or political views. The rapidly growing importance of science in the affairs of mankind increases the importance of the community of understanding.

"The ability of scientists all over the world to understand one another, and to work together, is an excellent instrument for bridging the gap between nations and for uniting them around common aims. We believe that working together in every field where international cooperation proves possible makes an important contribution toward establishing an appreciation of the community of nations. It can contribute to the development of the climate of mutual trust which is necessary for the resolution of political conflicts between nations, and which is an essential background to effective disarmament. We hope scientists everywhere will recognize their responsibility, to mankind and their own nations, to contribute thought, time, and energy to the furthering of international cooperation. . . .

"We call for an increase in the unrestricted flow of scientific information among nations, and for a wide exchange of scientists."

Certainly we do not believe that there are enough dentists in the world that any international relationship evolving from their research contacts can have great international consequence. Yet the relationship between nations is in part determined by the rapport between individuals within nations, and there are may instances where scientific contacts have provided important links in understanding. The British author, Charles P. Snow, has pointed out that, in the past, there have been scientific relationships in the fields of physical science, especially physics, mathematics, and natural sciences which are not possible today. However, there remains a good deal of international community of science in other fields, as biology.[3] He believes that the moral and intellectual leadership of science will pass to biologists and that it is among them that we shall find the Rutherfords, Bohrs, and Francks of the next generation.

In speaking on "The Moral Un-neutrality of Science," Mr. Snow also described some of the personal links within the international world of science which were possible back in the "twenties." There was a loyal Soviet citizen named Peter Kapitza, who worked in Rutherford's laboratory in Cambridge, England, for sixteen years. He became a member of the Royal Society and a fellow of Trinity College, Cambridge. He never gave up his Soviet citizenship and is now the director of the Institute of Physical Problems in Moscow. Through him, a generation of English scientists came to have personal knowledge of their Russian colleagues. These exchanges were then, and have remained, more valuable than all the diplomatic exchanges ever invented. Today, it would seem rather impossible for a physical scientist to be exchanged between the laboratories of the Soviet Union and one of the Western Allied countries. The development of atomic science and the political competition in the atomic and missile fields would make such an exchange akin to spying and treason. Fortunately, it is still possible for biologists, especially those in the health sciences, to work in each other's laboratories and exchange ideas across political barriers.

In the field of dental research, we now have several men working in laboratories in foreign countries, and there are a number of visiting dental scientists from other countries working in America. Exchange scientists have the opportunity of expressing views on world problems in a way that will be best understood under circumstances such that they will be accepted, not as political connivations, but as an expression of personal judgment. Although the area of special competence of scientists lies in the discovery of technical facts,[4] they too have a responsibility of voicing their own convictions on political and social problems. Naturally, a chemist's opinion on the social application of a chemical finding may be no more valid than that of a clergyman, but both are entitled to express these views in a democratic organization. In fact, it is their duty to share in the expression of national opinion. In an actively functioning international organization of dental scientists, not only would there be ample opportunity for exchange of scientific material, but the members would come to know each other's philosophical fiber, and there would be exchange of social and political ideas.

There are advantages at the professional level in increasing our personal contacts with other nations. Valuable research findings are coming from many foreign laboratories, and there are special advantages in conducting certain kinds of research in other lands. Examples of this which need little substantiation are the nutritional studies that have been done in India and the Malay Peninsula, studies on caries prevention with trace elements and phosphates being conducted in New Zealand and Peru, and studies on periodontal disease in Egypt, the Near East, and Asia. A second important area where we can gain information from the experience of other nations is in the socialization of dental practice. Each country is prone to believe that its own way of doing things is the best way. However, if one examines how dental care is provided to children in several European countries, it will soon become apparent that there are several different ways of providing better care than is given, on the average, in our country. We may not wish to accept any one European system of providing dental care to children or any one system of utilizing auxiliary personnel in dental practice (such as those of New Zealand and England); yet we are closing our eyes to progress if we don't examine the successes and failures of other nations in their approaches toward resolving these specific problems. Until good research methodology

is applied to our epidemiological and social science problems, it will be hard to remove national bias from our evaluation of the work of others.

Beyond the national and professional advantages of increasing our international relationships, there are many gratifications that come with personally knowing and becoming friendly with people from other countries. In living abroad, one soon learns that many of our stereotypes of foreign peoples are wrong, and it is also interesting to learn what other people think of themselves. Several years ago, Art Buchwald interviewed the British actor, Robert Morley, regarding differences between the Englishman and the American. Among his observations were that the British tourist always wants to be taken for British, whereas the American tourist is pleased when he is taken for a native. For the American living in England, there is a never ceasing amusement of observing how differently the two major English-speaking nations express themselves. As an illustration, I will quote two sentences exchanged between two Englishmen in a London pub:

FIRST ENGLISHMAN: "Awfully sorry to hear you buried your wife this morning, old boy."

SECOND ENGLISHMAN: "Yes, had to! Dead, you know!"

Having discussed some of the reasons for wanting a truly international organization, I would like merely to outline some of the problems entailed in attaining internationality. If the IADR is to be recognized as a truly international organization by other official bodies, then there are some conditions which must be met. On May 29, 1959, the Union of International Organizations requested the Director-General of UNESCO to consider drawing up an international convention which would set the conditions required of subscribing international, non-governmental organizations.[5] The conditions recommended include the following:

1. The membership must include representatives from at least six countries.
2. The aim must be international and non-commercial.
3. There must be international activity.
4. The administration must have international composition, and there should be a permanent secretariat.
5. Its funds must be substantially derived from more than one country.
6. Its activities must be recognized in one contracting State or enjoy consultative status with an intergovernmental institution (as UNESCO).
7. Its activities must be in harmony with the principles of the United Nations Charter and the Universal Declaration of Human Rights.

The draft of the Convention from which I have been quoting continues to describe the special benefits which would be expected to accrue to those associations which met the conditions, such as (1) being allowed the most favorable treatment which is accorded to national non-profit-making organization under the common law of the host country; (2) being given special consideration regarding passports, visas, foreign currency exchange, etc.; (3) being given as favorable rate of exchange as possible, as well as permission for free utilization of all funds and transfer of funds; (4) being exempt from restrictions relating to foreign labor, circulation of educational literature, copyright restrictions, etc.

How would these conventions affect IADR? It would be necessary that the officers

of the International Association be of international origin; the annual meetings would have to be held in different countries; its funds would have to be collected from and used in different countries; and the Association would have to have status in some established intergovernmental institution. I wish to report that answers I received to inquiring letters sent to friends in England, France, Germany, Switzerland, and the Scandinavian countries nearly all contained several conditions similar to these as being necessary if IADR is to expect to flourish as an international organization.

Let us consider for a moment the problems which would arise from holding the annual meeting of IADR in various countries. To list a few, there are the language problem and simultaneous translation, local printing and publishing problems, customs regulations, passports and visas, lack of authority over national bodies, national jealousies, difficulties in segregating international and local responsibilities in arranging meetings, problems in equitably selecting papers for presentation and location of these to the program, and many other minor things. There is little doubt, however, that all these problems can be met. The major item, so far unmentioned, is not met so easily; it is the cost of attending meetings which are far from home. I quote from a letter from Strasbourg, France: "It would be an excellent idea to give a broader scope to IADR. This would promote more international contacts in dental research. The only complication in attending meetings which are far away is that, in Europe, we have great difficulties in obtaining travel expenses from our universities." The matter of travel cost is serious, and perhaps it is the controlling factor in our final solution. Before we suggest a solution, I should like to mention some sources of funds.

On February 9, 1960, hearings began before the House Sub-committee of the Committee on Interstate and Foreign Commerce of the 86th Congress which dealt with the availability of foreign currency for use in international health research. Time will not permit a summarization of these hearings, but the testimony given indicated that funds approximating $3.8 million were available for foreign grants in 1960, and $4 million were the projected grant funds for (fiscal year) 1961. These funds went to foreign investigators whose proposals met carefully stipulated qualifications; they went to support interchange of highly qualified investigators in a "guest scientist" program, and they supported certain other specific activities, such as the establishment of foreign laboratories to study unique diseases. The transmission of scientific information by publication and by assembly is a legitimate and necessary cost of research and, if properly carried out, may receive certain federal support. At least this may be said: supporting agencies recognize that there is a need for international meetings, and they permit traveling expenses to be charged against research budgets.

In addition to sources of funds from our own federal agencies, such as described, there are *international* funds available for worthwhile scientific purposes. In 1958 the United States made available to WHO three hundred thousand dollars, and in 1960, another five hundred thousand dollars for specific studies related to developing research personnel and world co-operative research planning. Perhaps WHO or UNESCO could be induced to foster organized international dental research if our program were sound and our organization equitably distributed among nations.

Finally, commercial support must not be overlooked. In 1959, there were funds made available to IADR by the Colgate Company to support an International Conference on Oral Biology, and again in 1962, as you know, this company is supporting

a Second International Conference to be held in Bonn, Germany. I believe that periodic international meetings can continue to receive support by companies manufacturing dental products. I would recommend that we seek to get the *combined* support of groups of manufacturers of dentifrices, or therapeutic agents, or manufacturers of supplies and heavy equipment to sponsor periodic international meetings.

It would seem to me that the first step in solving the problem of internationality is to recognize that, inadvertently, during the growth of IADR in America, the American Division has swallowed up the International Organization. Although we call this session which is presently meeting the annual meeting of the IADR, is it not, in truth, the annual meeting of the American Division? For a combination of reasons—economic, political, and military—there has been a unilateral growth of our Association which almost certainly was not part of the plan that the founders had in mind. Our founders spoke of a growth of organized dental research in all countries of the world. As we meet here in Boston this week, there are a few attending members from other countries, who, by chance, were coming to America, but there are no official representatives from other continents. (Dr. Blackwood is representing the British Division at this session because he, too, had planned a trip to America that coincided with our meeting.) If we have no representation from abroad, if we never hold meetings abroad, and if we have none of our official staff from abroad, would it not be as logical to call this an "Interplanetary" association as an "International" one? If it takes us over forty years to get a meeting outside America, might we not have equal hope of getting a meeting in Mars within the next forty years? The solution, then, may require us to call these sessions of our "Divisional Meetings" and to follow through by injecting some life into the International Organization.

The next step has been expressed by a friend of mine in Birmingham, England:

"I think most of us, sooner or later, come to the conclusion that the ideal congress is one in which there is only one meeting going on at a time, with a small collection of papers and a firm hand kept on the timing. This, of course, means a great deal more selection in advance, but I would have thought, ideally, a small amorphous committee could have gone through the papers (their decision being final) and weeded them out until they got down to a few good and original ones in whatever field they might be, and that these were given."

If you will agree with this line of reasoning, then we may conclude that international dental research meetings could be considerably smaller than our present meeting and yet be profitable. This would lessen the transportation problem. Let us say that the United States and Canada would send, officially, only 5–10 per cent of its present number of participants and that Great Britain would follow this lead. Naturally, it would be expected that non-official members would attend these meetings of their own volition and participate in discussions. Perhaps the four Scandinavian countries and a newly formed west-central European division could be encouraged to co-operate and send well-selected representatives.

Does it seem possible that this would encourage growth also in the mid-European countries and in other continents? With a bit of organizing and a lot of hard work on the part of a devoted few, a high-quality meeting could be planned which would include the best papers from dental investigators throughout the world. These meetings

could be held in different countries year after year, as are other meetings of scientific groups. The officers of the International Association could be elected from nominations made by each division, with a rule that no president could come from the same country in successive years. We would end up, I am sure, with a smaller annual meeting, but it could be of high quality, and it could be truly international.

If you have gone along with these first two steps toward internationalism, we still have to solve important problems in the mechanics of bringing these changes about. Let me read from a letter from Helsinki, Finland:

"The complications of the new arrangement would mainly be financial and providing adequate and equally distributed support for the meetings. In addition, the organization would be heavier than at present. A fusion of the FDI (Fédération Dentaire Internationale) and the IADR might offer a solution; an existing international organization with a world-wide network could *perhaps* include a pure and exclusive research group. Personally, I have always found the FDI organization a real mammoth, and just for this reason, a re-organization including a fully independent partner might be possible."

It is significant that another outstanding dental investigator should write almost identical comments from Stockholm, Sweden, and I quote:

"Finally, we have to cope with organizational problems, which would also be of a prestige nature for a good part. Good co-operation with the FDI would probably be necessary. The ORCA and the ARPA should preferably merge into a European branch of an international dental research association, and it would require a good deal of effort to perform such a fusion.

"I think the first step would be to win the [support of] IADR, the FDI, and especially its Scientific Commission, and the most important regional dental research associations for your plan. With the [present] chairman of the Scientific Commission, I think there is a good chance of obtaining the support of FDI. It would also be important to place a scientifically qualified and authoritative person as Chief Dental Health Officer in the WHO in Geneva, where the dental branch could be developed into something quite different from its present status. The WHO has a newly developed scientific program involving, among other things, the support of international scientific exchange."

He concludes his letter with: "I wish you all success and shall be prepared to support your plans in my part of the world as much as I can."

I believe these last two letters from which I have quoted reveal important aspects of necessary mechanisms which must be utilized in any strengthening of an international dental research organization. Specifically, we must share responsibility and authority with other research organizations that already exist. If it appears that we are trying to swallow them up rather than merge with them, we can expect little desire for co-operation. The by-laws and regulations of each of the national organizations for dental research can be evaluated, and the best objectives can be selected for a revised international charter. Unless something of this kind is done, there is not likely to be a growth of internationalism in dental reesarch. Unless we, as Americans, take the initiating action necessary to bring about such changes, the IADR can never be developed into a newly structured international organization, for other countries are never sufficiently represented in our meeting to influence a change.

In summary, then, I have said that we need to recognize the limited international scope of our present organization. Recognizing that we are more national than international, we must examine the desirability, the advantages, and the difficulties of becoming truly international. I have discussed some ways of overcoming the difficulties of holding international meetings and have reviewed some mechanisms that would be useful in reorganizing our association. I personally believe we should have a worldwide organization for dental research. To me, the advantages far outweigh the disadvantages and the sacrifices that we would have to make. Should you, as North American members of IADR, indicate that you are interested in strengthening our international bonds, I would be willing to devote considerable time during my presidential term collecting necessary information, so that, at next year's meeting, we would be in a position to make firm decisions. The decision rests with you.

REFERENCES

1. GIES, WILLIAM J. *J. D. Res.*, **6**:101, 1924–26.
2. "News of Science," *Science*, **128**:1071, 1958.
3. SNOW, CHARLES P. "The Moral Un-neutrality of Science," *Editorial Sci.*, **133**:255, 1961.
4. "Between Two Extremes," *Editorial Sci.*, **131**:1013, 1960.
5. *Intern. Assoc.*, **11**:510, 1959.

International Association for Dental Research

Abstracts of Papers Presented at the 39th General Meeting

STATLER HOTEL, BOSTON, MASSACHUSETTS, MARCH 23–26, 1961

*Compiled by FRANK J. ORLAND, EDITOR**

I. CLINICAL STUDIES. MARCH 23, AFTERNOON, SECTION A

1. A NON-METALLIC STRESS-BREAKER IN COMPLETE AND PARTIAL DENTURES.—*Herbert M. Parker, New York, New York.* Since resorption of the supporting structures is commonly related to the action of deleterious or excessive forces transmitted through the prosthesis, the recognized need for softening the blow to the edentulous mandible is not new. The use of velum rubber applied to the lingual flanges, large tori, and spiny bone processes was followed by the use of more versatile acrivelum and, most recently, by soft cushion linings. This study dealt with the reduction of the impact force to the supporting tissues of a complete denture. No change in impression making or record taking was involved. The stress-breaker consisted of a resilient resin filler sandwiched between layers of denture acrylic resin; at no time did the soft acrylic resin contact the supporting basal tissues. In such a manner, adjustments of the denture tissue surface could be made as usual, without the clinical disadvantages inherent in soft acrylic cushions. This study was divided into two phases: (1) laboratory and (2) clinical. Samples of four commercial resilient resins were subjected to elastic deformation tests ranging from 10 to 120 psi, in order to accommodate the general range of masticatory pressures. The results were charted and graphed. In each case the resilient resin selected was placed between two layers of hard acrylic resin in a lower denture, for a patient whose lower ridge presented chronic difficulties commonly seen by a prosthodontist. Concurrently, a conventional duplicate lower denture was made to articulate with the upper, so that a clinical comparison could be made.

2. A NEW TECHNIQUE FOR MEASURING TOOTH MOBILITY.—*Hyman J. V. Goldberg, Eastman Dental Dispensary, Rochester, New York.* Mobility of teeth is an excellent index of periodontal status. However, the accurate measurement of such mobility has presented many problems. Foremost of these problems is the attachment of the measuring device to the teeth in question. We have developed a carriage device which can be used with the meter utilized in the Mühlemann technique of measuring, as well as with other measuring techniques. This carriage device abates many of the problems associated with measuring and allows the simultaneous measurement of maxillary and mandibular teeth. The carriage device is held in the oral cavity by engaging the occlusal surfaces of both the maxillary and the mandibular posterior teeth with "quick-cure" acrylic or impression plaster. A microscope stage was adapted to become the adjusting mechanism of our device. This greatly facilitates all movements in the horizontal placement of the gauge needle. A vertical rod controls the vertical move-

* The members of the Program Committee, James A. English, Holmes T. Knighton, S. J. Kreshover, and Dan Y. Burrill, as well as the Program Chairman of the Dental Materials Group, gave invaluable assistance in the preparation of these abstracts.

ment so as to allow measurement of both the maxillary and the mandibular teeth. The carriage is guided around the mouth with a specially designed semicircular platform which is attached to the holders of the acrylic occlusal keys. Measurements of tooth mobility, made with this apparatus on 80 teeth, indicated that our technique produces accurate and reproducible measurements. This technique can be learned readily and can be applied to clinical studies where precise mobility measurements are desired.

3. (Not presented.)

4. Change in Enamel Surface Caused by Oxyphosphate Cement.—*Robert Wade Seniff, Kansas City University School of Dentistry.* The effect of zinc phosphate cement on the surface of enamel has been a subject of controversy. This study was designed to demonstrate the surface change and to determine whether a single application of 8 per cent stannous fluoride would influence the change. Lines were scribed on the buccal surface of 32 freshly extracted bicuspid teeth for orientation. The teeth were polished prior to examination. A collodion replica of this surface was taken as a control. One-half the control surface was coated with collodion, and the tooth was immersed in 8 per cent stannous fluoride for 4 minutes and then air-dried. The collodion was then removed from the enamel. Phosphoric acid (cement liquid) was applied to the entire surface for 5 minutes. Thin, medium, and thick mixes of zinc phosphate cement were applied to the surfaces for 24 hours and then removed. Replicas of all surfaces were then taken. The results showed that the phosphoric acid severely etched the enamel surface. The thin mix also etched the surface. The medium and thick cements produced slight etching. The surfaces treated with stannous fluoride showed a marked reduction in etching. Little difference was found between surface changes in carious and non-carious teeth.

5. The Acidity of Zinc Phosphate Cement after Setting.—*Fred W. Cook and George H. Horne, University of Kansas City School of Dentistry.* A spectrophotometric method of determining the pH of solid films was applied to the problem of zinc phosphate cement. Color changes of an indicator-dye system, incorporated into the cement, were measured in a spectrophotometer.* The color changes were converted to pH's from a standard curve developed for the indicator-dye system. Replicate samples of two commercially available zinc phosphate cements were measured for pH changes, after setting, at increasing time intervals from 15 minutes to 30 days. The mean pH of both brands was below 5.0 after 30 days. These findings confirm previous reports that the powder does not react completely with the liquid and that residual acidity, in some form, is present in set zinc phosphate cement.

6. A Modified Analysis of Variance in a Clinical Tooth-brushing Experiment.—*John W. Fertig and Neal W. Chilton, Columbia University, New York, and Temple University, Philadelphia, Pennsylvania.* The clinical effectiveness of an electric toothbrush was compared with that of a conventional toothbrush. The study group, consisting of 30 first-year dental students, was divided into two subgroups by random alternation, with one subgroup using the electric brush on the maxillary teeth and the conventional brush on the mandibular teeth, and the other subgroup using the brushes in reverse order. This alternation rendered the brush differences independent of jaw differences. Following an initial examination, the 30 subjects were given the brushes and were examined weekly for 8 weeks. Since the fundamental interest in the experiment was in the comparison of the brushes, a complete analysis involving all the criteria was not necessary—a complete analysis would require thirteen terms. The brush effects were calculated directly by using appropriate differences of the measurements for the various pairs of individuals and time periods. The analysis was then performed on the two-way layout of differences. The primary brush effect was measured by comparing the change from the control level to the average level

* Beckman Model B.

during the 8 weeks of brushing. The comparison of the changes during the brushing period afforded an auxiliary comparison of the two brushing techniques. The division of the whole time span, including the control level, into two parts could be accomplished without greatly complicating the analysis. No statistically significant difference in the clinical effectiveness of the two brushes as measured by the PMA Index was found.

7. Sequential Analysis in Clinical Dental Research.—*Neal W. Chilton, John W. Fertig, and Austin H. Kutscher, Columbia University, New York, New York.* During World War II the need for determining as rapidly as possible which of two products was better gave impetus to the development of sequential analysis. By this method, a decision as to statistical significance or non-significance can, on the average, be arrived at with a smaller sample size than by the conventional method (fixed sample size). In sequential analysis the investigator inspects the results continually rather than waiting until the study is completed to make his decision. Sequential analysis was employed in a study of the comparative clinical effectiveness of 5 per cent tetracaine in two different ointment bases as a topical anesthetic on oral mucous membranes. The calculation of the critical zones was presented according to various criteria for a one-sided and a two-sided test. The data from the experiment yielded statistical significance with forty observations (fourteen untied pairs) in the one-sided test. Significance was not obtained in the two-sided test. The relative merits of the sequential method and the conventional fixed-sampling methods are discussed.

8. Effects of Oral Prostheses on the Production of Speech Sounds.—*Alexander L. Martone, Norfolk, Virginia.* The purpose of this study was to investigate the effects of certain alterations of the palate and tooth positions on the production of speech. This study was conducted to test the quality and articulation of consonant and vowel sounds as spoken by 50 normal subjects with six artificial variations in the palates and dental arches. The stimulus material was recorded with these palatal forms in place in random order. The recordings were edited, and stimulus ratings were made by four panels of judges by a psychophysical method. The responses of the judges were treated by an analysis of variance. The statistical analyses revealed the following observations: (1) there were no significant differences in the vocal quality of the vowel; (2) there were significant differences in articulation of speech with various alterations of the palate and teeth; (3) the artificial palates affected the utterance of a sentence differentially. The results indicated that variations of the palate and position of the teeth in artificial appliances did influence the production of speech, primarily from the standpoint of articulation of a sentence and, to an insignificant degree, the quality of a vowel. It was concluded that the phonetic factor should be treated with the same scientific approach that is applied to the mechanical and aesthetic factors in complete denture construction.

9. Pulp Reaction to Five Filling Materials Related to Cavity Depth.—*David F. Mitchell, Michael G. Buonocore, and Shirley Shazer, Indiana University School of Dentistry and Eastman Dental Dispensary.* In 3 *Rhesus* monkeys, 18, 33, and 36 months old, class V cavities were prepared in 54 teeth under uniform conventional conditions except that the depth varied. Zinc oxide–eugenol, zinc oxide–eugenol plus croton oil, silicate,* silicate with acid diluted by one-third, and an experimental silicate were used as fillings. Fourteen deciduous and 20 permanent teeth were extracted after 7 days, and 20 permanent teeth were extracted after 42 days. Extracting procedures insured proper pulp fixation, and serial sections of every tooth were prepared. About 120 sections of tooth were screened; 60 good sections of tooth were studied. All cavities involved cut dentin. The floors of 18 cavities were thinner than 1 mm.; 36 were thicker. When the cavity floors were thicker than 500 μ, all reactions were mild. Reaction to all materials (except zinc oxide–eugenol) increased

* L. D. Caulk Company, Milford, Delaware.

as floor thickness decreased to 50 μ, or pulp exposure. The need for serial sections in such studies was demonstrated in many ways; e.g., in one tooth, mild reactions beneath the cavity where the floor was thicker were offset by severe reactions beneath the same cavity where near exposure existed. With the exception of the consistently mild zinc oxide–eugenol, no differences between the reactions to the materials were demonstrated.

10. Calcified Material in Human Gingival Tissue.—*Bernard S. Moskow, Fairleigh Dickinson University School of Dentistry, Teaneck, New Jersey.* A previous study demonstrated tissue changes in the sulcular wall of gingival tissue following instrumentation. In this experiment calcified material was observed in the corium of these gingival sections with great frequency. Further study of this gingival tissue was undertaken to determine the nature of these calcified bodies and their possible mode of origin. While some of the calcified material resembled dentin and cementum histologically, other specimens demonstrated inclusions which possibly were particles of calculus or filling material. Similar fragments were also observed in deep splits in the sulcular wall of the gingiva created during a previous scaling. Some of the gingival calcifications appeared well encapsulated, with no inflammatory response to the material. It was postulated that much of the calcified material seen in gingival biopsies was material removed from the tooth surface during instrumentation and displaced into the connective tissue of the gingiva through slits created in the gingival wall.

11. Influence of Occlusal Wear and Age on Dentin and Pulp.—*George G. Philippas, Athens, Greece.* Effects of age and occlusal wear on dentin apposition and pulp-chamber shrinkage were observed in 168 sound permanent first molars from both sexes aged five to seventy years. The teeth were drawn both from contemporary individuals in vivo, and ancient skeletal material in situ showing degrees of occlusal wear O to V inclusive (Philippas, *J.A.D.A.*, **45**:446, 1952). Measurements of all teeth were taken on enlarged X10 radiographs under identical conditions from the buccal aspect, using eight standardized indices and based on two axes: (1) vertical from the cemento-enamel junction at the center of the occlusal surface to the gingival extremity at the bifurcation of the roots and (2) perpendicular to axis 1, bisecting it at the minimum height of the pulp chamber. These measurements were tabulated separately for contemporary and ancient material by age groups and degree of occlusal wear and then analyzed statistically. Irrespective of degree of occlusal wear, the dentin at the roof of the pulp chamber showed no significant increase over the age range studied. The medial and distal walls of dentin showed slight increase with age, and that on the floor showed the greatest. Results indicated that, regardless of occlusal wear, dentin apposition continues throughout the life of the sound tooth, though varying in amount over different apposition walls and slowing down with increasing age. This continuous apposition with age, not wear, appeared to be responsible for pulp-chamber shrinkage.

12. A Pinhole Camera for Radiation Research in Dentistry.—*Albert G. Richards, School of Dentistry, University of Michigan.* A pinhole camera was described which produces simultaneously, radiographs and photographs of any object that emits X-radiation and reflects visible light from its surface. This camera is ideally suited for radiation research in dentistry because both the patient and the X-ray apparatus emit X-radiation and reflect visible light while the patient is examined with X-rays. The camera is essentially a metallic box containing two concentric pinholes in its front surface, and a combination photographic and radiographic film holder forms its back surface. The combination film holder is devised so that light, which enters the photographic pinhole, will expose the photographic film while the scattered X-rays that enter the radiographic pinhole will expose the radiographic film located between two intensifying screens. Examples were presented of the two types of pictures that are produced with this camera. By viewing the superimposed photograph and radiograph,

the location of the invisible sources of X-radiation portrayed in the radiograph can easily be oriented with respect to the visible details in the photograph.

13. EXAMINER VARIABILITY IN THE DISCERNMENT OF OCCLUSAL TRAITS AND ORAL HABITS.—*D. R. Paulson and D. R. Porter, University of Oregon Dental School, Portland, Oregon.* Following two trial runs to standardize the three examiners, a field study was initiated. A random sample of 38 boys and 38 girls was selected from the 1,000 Portland school children of the main study for blind triplicate examinations, in order to test examiner variability in the discernment of occlusal traits and oral habits. The findings for each examiner were normalized and subjected to analyses of variance, testing at the 1 per cent significance level. The variability among the examiners in certain groups of traits, i.e., anterior relationships, availability of space, and occlusal relationships, was not significantly different. Differences between examiners in discerning oral habits and cross-bites were significant. First-order interactions were tested for each of the groups of traits and variables. Significant differences between examiners were found for anterior relationships, occlusal traits, and oral habits. The differences between sexes were not significant; differences between traits were significant.

II. PATHOLOGY. MARCH 23, AFTERNOON, SECTION B

14. THE ROLE OF MECHANICAL STRESS IN EXPERIMENTAL LATHYRISM.—*George A. Krikos, School of Dentistry, University of Pennsylvania, Philadelphia.* These studies were conducted for the purpose of obtaining knowledge of the role of mechanical stress in the manifestation of the connective tissue changes seen in experimental lathyrism. Weanling male rats were divided into four groups of six rats each. All animals were pair-fed and received fox chow;* 0.4 per cent beta-aminopropionitrile fumarate was added to the diet of the experimental rats. All animals had their maxillary right molars removed 24 hours before they were placed on their respective diets. One control and one experimental group were sacrificed after 7 and 14 days on their diets. Sections prepared from the mandibles of all rats were stained with hematoxylin and eosin, silver stain, van Gieson, and Masson's trichrome stain, and examined with the light microscope. The periodontal structures of the left mandibular molars of the experimental animals were under mechanical stress and exhibited marked alterations. The periodontal membrane showed round or oblong areas of an amorphous eosinophilic material surrounded by palisading connective tissue cells. The alveolar bone was osteoporotic. The periodontal structures of the right mandibular molars of the experimental rats were not under mechanical stress and did not exhibit any abnormalities, being, in all respects, similar to the corresponding structures of the control animals. These observations suggest that mechanical stress plays an important role in the manifestation of the changes of lathyrism as seen at the level of the light microscope.

15. FIBROPLASIA AND HYDROXYPROLINE DETERMINATIONS IN POLYVINYL SPONGE IMPLANTS IN DIPHENYLHYDANTOIN SODIUM-TREATED RATS.—*S. Miles Standish and Patricia G. Clark, Indiana University School of Dentistry, Indianapolis.* Adult male albino rats, 60 days of age, were given sodium† (25 mg/day) by stomach tube for 8 days prior to subcutaneous implantation of measured cylinders of polyvinyl sponges.‡ Control animals received the diluent, 10 per cent gum acacia, by stomach tube for the same period. At the time of implantation the animals' backs were shaved and 6 previously prepared cylinders (10 mm. in diameter × 15 mm. in length) of polyvinyl sponge were introduced into separate subcutaneous pockets through incisions placed on either side of the mid-line. Treatment with diphenylhydrantoin sodium or the control solution was resumed on the day following implantation for 6 and 14 additional

* Purina Fox Chow.

† Dilantin sodium, Parke, Davis & Co.

‡ Ivalon®, Simonize Co., Chicago, Illinois.

days. At sacrifice, the 3 sponges from the right side of the back of each animal were fixed in Bouin's solution or 10 per cent formalin and prepared for histologic examination. Estimates were made of the extent of connective tissue invasion of the interstices of the sponge by means of a micrometer eyepiece. Sponges removed from the left side of the animal's back were dissected free from the investing capsule, weighed, and subjected to hydroxyproline determination according to the method of Neuman and Logan. Animals receiving sodium and sacrificed 6 days following sponge implantation demonstrated significantly greater hydroxyproline values than the controls. At 14 days, hydroxyproline values in the sodium group were less than the controls, a trend supported by the histologic studies.

16. Separation of Oral Epithelium for Chemical Analysis.—*Alvin L. Morris and Eva Mertz, School of Dentistry, University of Pennsylvania, Philadelphia.* Investigations were carried out in an attempt to develop a method whereby oral epithelium of small biopsy specimens could be separated from underlying connective tissue for chemical analysis. Seven techniques described as being successful with skin were not effective with oral mucosa. Felsher's method, in which skin is exposed to 2 M sodium thiocyanate for 10 minutes, was found to be effective with oral mucosa. In an attempt to minimize the disturbance to the chemical constituents of epithelium, investigations were carried out to determine the most favorable combination of the following variables known to affect the technique: tissue size, concentration of NaCNS, time, and temperature. Human gingivectomy specimens were placed in room-temperature tyrode solution for up to 2 hours. Either immediately thereafter or following 1–5 days' storage at approximately —20° C., the tissues were cut into 3 × 4-mm. pieces and exposed to NaCNS. Oral epithelium could be easily dissected in one sheet, free of connective tissue, following 11 minutes' exposure to 0.9 M NaCNS at 2° C. Chemical analyses of hamster cheek pouch treated in the described manner revealed no significant differences as compared with untreated pouches, in levels of protein, soluble-SH, hexokinase, and dehydrogenases for glucose-6-phosphate and phosphogluconate.

17. Effects of Cortisone on Experimental Carcinogenesis in the Hamster Submandibular Gland.—*W. R. Sabes, A. P. Chaudhry, and R. J. Gorlin, University of Minnesota, Minneapolis.* Seventy-four male golden Syrian hamsters were divided into four groups of 20, 18, 18, and 18 animals each. The animals of Group I were anesthetized with Pentobarbital sodium,* the right submandibular salivary glands surgically exposed, and the glands injected with 0.05 cc. of 0.025 per cent cortisone† in saline (1.25 γ). This was immediately followed by injection of the same glands with 0.05 cc. of 0.5 per cent 7,12-dimethylbenz (α) anthracene in liquid petrolatum (250 γ). Group II animals were similarly treated by injecting 0.05 cc. of the cortisone vehicle, followed by carcinogen. Group III was injected with 0.05 cc. of physiologic saline followed immediately by 0.05 cc. of carcinogen. Group IV was treated with 0.1 cc. of 0.25 per cent carcinogen alone. One-half of each group was treated in the A.M. (9–12) and the other half in the P.M. (2–5). Since the noncarcinogenic properties of liquid petrolatum had been repeatedly established in this laboratory, no control for liquid petrolatum was employed. Eighteen weeks after the injection date the animals were sacrificed with lethal doses of Nembutal. The tumors were removed and fixed in Zenker's solution. Histologic sections of all tumors were prepared and stained with hematoxylin and eosin. Microscopic analysis of the tumors revealed that the group of animals treated with cortisone developed fewer sarcomas than did the other groups. However, this intergroup difference between the control and experimental animals was not statistically significant ($p = 0.15$). When the data were analyzed statistically by morning and afternoon grouping, it appeared that such a difference was significant ($p = <0.05$). When the animals presenting sarcoma were noted according to time of

* Nembutal®, Abbott Laboratories, North Chicago, Illinois.

† Cortogen®, Schering Corp., Bloomfield, New Jersey.

injection, irrespective of group classification, it appeared that the periodic distribution of induced cancers had completely obscured the effects of cortisone. This factor may be responsible for conflicting reports in the literature of the effects of cortisone on carcinogenesis.

18. GENETIC AND CHROMOSOMAL ANALYSIS OF A NEW ORAL SYNDROME.—*Robert J. Gorlin, University of Minnesota School of Dentistry, Minneapolis.* A genetic investigation was carried out on a new oral syndrome consisting of pseudo-clefts of the upper lip, palate, and tongue due to hypertrophied frenuli. Associated with these abnormalities are aplasia of alar cartilages, oligophrenia, familial trembling, and true cleft palate. Genetic studies revealed that the syndrome was limited to the female sex, and, from the data available, it appears that the condition may be lethal in the male. This syndrome was first described by Papillon-Leage and Psaume in 1954, but this family is probably the first to be analyzed in this country. Although there is good penetrance, there is variable expressivity. In contrast to the views of Papillon-Leage and Psaume, who viewed the symptom complex as recessive, our study revealed a well-defined dominant inheritance pattern. Chromosomal studies, i.e., counting and sex determination, were carried out without abnormal findings.

19. ORAL AND SALIVARY GLAND CHANGES IN SJÖGREN'S SYNDROME.—*Irwin I. Ship, University of Pennsylvania School of Dentistry and the Philadelphia General Hospital.* Sjögren's syndrome, a triple-symptom complex, consists of keratoconjunctivitis sicca, xerostomia, and rheumatoid arthritis. Xerostomia has been reported to be present in 55 per cent of Sjögren's cases (*Svenska Lakartidningen,* **46**:2413, 1949), and in 83 per cent of advanced cases reported by Gifford (*Arch. Ophthal.,* **30**:207, 1943). Maintenance of normal salivary flow involves both major salivary glands (parotid, submaxillary, and sublingual) and the small mucous and serous glands in the oral mucosa. A history of increased fluid intake and dryness of the mouth may not necessarily be substantiated by clinical evaluation, and major salivary gland involvement may be present in the absence of xerostomia. A technique for the evaluation of subclinical salivary gland changes may have qualitative, as well as quantitative, value in patients with keratoconjunctivitis sicca. With minor variations in the technique as reported by Rubin and Blatt (*Univ. Michigan Med. Bull.,* **21**:57, 1955), secretory sialography was performed on 52 patients. Thirty-one patients had Sjögren's syndrome (not less than 2 of the 3 symptoms), and 21 patients had rheumatoid arthritis with no other symptoms of the syndrome. Criteria for the evaluation of the radiographs were modified from Payne (*Lancet,* **1**:348, 1933), Parrott (*Ann. d'oculist,* **187**:898, 1954), and Blatt (*Ann. Oto., Rhin., and Laryng.,* **65**:295, 1956). All patients with Sjögren's syndrome, with the exception of one, showed abnormal parotid changes. Two patients demonstrated punctate sialectasis, and 13 revealed changes of punctate sialectasis with intermediary duct involvement. Seven patients demonstrated globular sialectasis, and 8 were characterized as having cavitary or destructive sialectasis.

20. PRELIMINARY REPORT OF RESPIRATION CHANGES IN RAT GLAND TISSUE AS A RESULT OF LIGATION.—*J. H. Manhold, Jr., T. E. Bolden, and M. L. Hall, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey.* The exorbital lacrimal gland of the rat exhibits many similarities to the salivary gland and is more advantageous for study. A normal respiratory quotient ($Q_{O_2} = 0.41 \pm 0.14$) was established for exorbital lacrimal gland tissue of 150-day-old male Wistar rats by means of a microrespirometer technique (Manhold, Bolden, and Katz, 1959). Excretory ducts were then ligated, and the animals killed at 1, 2, 3, 7, and 14 days, 1 month, and 4 months after ligation. The duct-ligated glands were removed, placed in Tyrode's solution, and divided into two parts. One half was fixed for microscopic study, and the other half was placed in microrespirometers for Q_{O_2} determination. Results were: 1 day (11 glands)—0.36 ± 0.59; 2 days (9 glands)—0.34 ± 0.22; 3 days (8 glands)—

0.70 ± 0.08; 7 days (12 glands)—0.64 ± 0.53; 14 days (11 glands)—0.50 ± 0.14; 1 month (10 glands)—0.29 ± 0.25; 4 months (10 glands)—0.30 ± 0.19. These results corresponded with the observations made under the microscope. It was tentatively concluded that duct ligation caused an initial drop in the respiration of glandular tissue, followed on the third day by an increase. A constant Q_{O_2} value, which was lower than the initial value, was reached when the gland histologically showed more scar tissue and less parenchyma.

21. Mechanism in the Pathogenesis of Amyloidosis.—*Valy Menkin and Viola D. Flanagan, University of Kansas City School of Dentistry, Kansas City, Missouri.* The pathogenesis of secondary amyloidosis is still not resolved. Earlier studies by one of us (V. Menkin, 1958 and 1960) indicated that repeated subcutaneous injection of the canine euglobulin fraction, necrosin, into rabbits was frequently accompanied by large deposits of amyloid, particularly in the spleen, kidney, and liver. The concentrated diffusate of canine exudate, also containing the growth-promoting factor of exudate, following its repeated injection into rabbits was likewise accompanied by extensive deposits of amyloid in the spleen, kidney, and liver. Several intravenous injections of necrosin were followed by the formation of pre-amyloid material in cells of the reticuloendothelial system, particularly in the spleen and even in the liver, as indicated on Congo red- and PAS-stained preparations. The view was advanced that the liberation of necrosin, or of a diffusible component in close association with necrosin, pentetrated from the site of inflammation into the systemic circulation to reach target cells in the reticuloendothelial system, which in turn, as a result of their cellular activity, formed amyloid. The present studies following repeated subcutaneous injections of rabbit necrosin into rabbits likewise indicated the formation of pre-amyloid material in and around reticuloendothelial target cells. It is of interest to note, however, that in rabbits the albumin–pseudoglobulin fraction of rabbit exudate injected subcutaneously only 17 times during a period of about 1 month was accompanied by a very large formation of amyloid-like material in and around reticuloendothelial cells. The deposits appeared to be much more pronounced than when rabbit euglobulin or necrosin was employed. The precise factor or mediator responsible for the stimulation of amyloid formation is under further study.

22. Oral Cytology for Detecting Incipient Mouth Cancer.—*Henry C. Sandler, Veterans Administration Hospital, Brooklyn, New York.* Twelve Veterans Administration Hospital dental services have combined in a co-operative study to evaluate the practical application of oral cytology to the detection of early mouth cancer. Scrapings of every oral abnormality noted at routine examination, whether or not cancer was suspected, were obtained for cytologic study. Biopsies were obtained wherever indicated. At the end of the first year of study, scrapings of oral lesions from 841 patients had been examined. Biopsies from 218 patients were correlated, and cancer was present in 114 patients. The correlation between cytology and biopsy, independently determined, was of the order of 90 per cent. Of particular significance was the finding of 14 cases of asymptomatic, clinically unsuspected cases of mouth cancer which were not scheduled for biopsy and were uncovered by cytologic examination. Subsequent biopsy confirmed the cytologic findings. Seven of these were intra-epithelial cancers. The value and reliability of cytology for the detection of incipient asymptomatic mouth cancer was demonstrated.

23. Effects of Tween 60 upon Experimental Oral Carcinogenesis in the Hamster.—*Stephen F. Dachi, Indiana University, Indianapolis.* Studies were carried out on the role of polyoxyethylene sorbitan monostearate* as a promoting agent in 7,12-dimethylbenz-(α)-anthracene (DMBA) induced carcinogenesis of the hamster cheek pouch. DMBA in concentrations of 5 and 2 per cent was dissolved in Tween 60 and applied to the cheek pouch for varying intervals. The effects were compared with

* Tween 60.

those produced by using a mineral-oil vehicle for the carcinogen. The reactions of skin and pouch mucosa to Tween 60 were also compared. With 2 per cent DMBA dissolved in Tween, the latent period for tumor appearance was shortened by 2–3 weeks and decreased the life-span of the tumor-bearing animals. In a 5 per cent concentration it exhibited marked systemic toxicity. In this group the latent period for tumor appearance was unchanged, but the animals died shortly after the appearance of the initial tumors. Autopsy revealed non-specific degenerative changes in the liver. In a group receiving 5 per cent DMBA in Tween 60 in a dose which is subcarcinogenic with a mineral-oil vehicle, the toxic effects were less marked, and a few malignant tumors were produced. Applications of Tween 60 to skin resulted in a marked thickening of the prickle-cell layer and mild hyperkeratosis. No such effects were obtained in the cheek pouch.

24. Carcinogenesis and Mechanical Irritation in the Cheek Pouch of the Hamster.—*Grete Renstrup, Jerome Smulow, and Irving Glickman, Tufts University School of Dental Medicine, Boston, Massachusetts.* Two series of experiments were arranged to investigate the effect of mechanical irritation upon chemically induced carcinogenesis in the cheek pouch of the golden Syrian hamster (*Cricetus auratus*). Fifty-four 3-month-old female animals divided into 6 equal groups were used. A 0.5 per cent concentration of 9,10-dimethyl-1,2-benzanthracene in an adhesive vehicle was applied to the cheek pouch 3 times per week to 3 groups of animals throughout the experimental period, which extended from 4 to 24 weeks. In four groups, a twisted wire ligated around the lower first molar and projecting into the cheek pouch served as a local irritant. Gross and microscopic observations indicated the following: mechanical irritation alone resulted in persistent ulceration; by the eighteenth week, carcinoma was observed in all animals treated either with the carcinogen or with the carcinogen–mechanical irritation combination. The first series was conducted to determine the tissue response to the carcinogen, mechanical irritation, and the vehicle. The second series of 140 animals is being confined to comparing chemically induced carcinogenesis with and without mechanical irritation.

25. Cytodiagnosis of Oral Lesions Using Fluorescence Microscopy.—*C. W. Conroy, University of Texas Dental Branch, Houston.* The Papanicolaou method of cytodiagnosis, used successfully as an adjunct for the detection of oral carcinomas, is time-consuming and requires highly trained personnel. A pilot study was undertaken to determine whether a fluorescence technique might be useful for screening or diagnosis of oral lesions. Smears were made from normal areas of oral mucosa for preliminary study to establish a base line for future observations. Material for diagnostic evaluation was obtained from benign intraoral lesions which were classified as chronic irritations and ulcerations and from malignant oral neoplasms. Smears were stained for fluorescence study by the method described by von Bertalanffy, Masin, and Masin. Comparative smears were stained by the Papanicolaou method and histopathologic specimens were obtained for corroboration. The results of study of normal oral mucosa were in agreement with those from previously reported studies. Diagnosis of lesions due to chonic irritation was impractical without the clinical description of the lesion, since only relatively mature cells were usually observed. Detection of cells exhibiting features of malignancy was facilitated by the intense orange fluorescence of the ribonucleic acid in the cytoplasm, which served to attract the observer's attention. Only gross morphologic criteria of malignancy were discernible. Malignant cells were demonstrated in all cases of carcinoma by fluorescence and the Papanicolaou technique. The fluorescence method may be applicable as an aid in the evaluation of oral lesions and warrants further appraisal.

26. Epithelial Tumors Arising from Intraoral Minor Salivary Glands.—*R. A. Vickers and A. P. Chaudhry, University of Minnesota.* Information on the clinical behavior, treatment, and prognosis of tumors arising from the intraoral minor salivary glands is sparse and conflicting. This is largely due to the fact that such tumors are

relatively uncommon, and this could account for only a few well-documented reports available in the literature. The present investigation was based on 94 cases of such tumors collected from several Twin Cities hospitals, the largest number in a series that has ever been reported. Data on age and sex of the patients, duration and location of the tumors, the type of tumors, their treatment and prognosis were analyzed. These findings were compared with those of over 1,400 cases collected from the English literature published from 1927 to 1960, enabling the authors to draw valid conclusions based on a reliable sample size and thus resolve some of the conflicting views. Benign mixed tumors were the most common type, and the next in order of frequency was the cylindromatous variety of adenocarcinomas. The palate was the site of predilection for both benign and malignant tumors. Benign mixed tumors and low-grade mucoepidermoid tumors occurred in patients, on an average, 10–15 years younger than those with malignant tumors. In terms of their ability to recur and metastasize, the conventional type of adenocarcinomas were the most malignant, followed by cylindromatous adenocarcinoma and high-grade mucoepidermoid tumors.

III. ANATOMY AND HISTOLOGY, MARCH 23, AFTERNOON, SECTION C

27. CULTIVATION IN VITRO OF ADULT HUMAN SKIN AND ORAL MUCOSA ON GELATIN FILM.—*Jerome B. Smulow, Robert Rustigian, and Mauray Tye, Tufts University Schools of Dental Medicine and Medicine, Boston.* One hundred and sixty specimens of human skin were obtained from 58 adult donors, and 40 specimens of oral mucosa were taken from the lower labial region of 22 adult donors. Gelatin film and plasma clot were compared as substrates for the cultivation of these tissues. Zones of epithelial-like cells developed from single fragments of adult human skin and oral mucosa on gelatin film. The initial appearance of these cells was somewhat slower on gelatin film than in plasma clot, but subsequent migration or growth was comparable on both substrates. Fibroblast-like cells were rarely observed in skin cultures on gelatin film but appeared in the majority of plasma-clot cultures. Cultural characteristics of adult human skin and oral mucosa showed certain differences. The most striking differences were the absence of development of fibroblast-like cells in the mucosal cultures and the luxuriant outgrowth of mucosal cells on glass surface. Skin fragments, under our experimental conditions, showed little or no outgrowth on glass.

28. STORAGE METHODS IN TOOTH TRANSPLANTATION.—*Richard J. Coburn and Basil L. Henriques, Tufts University School of Dental Medicine, Boston.* One facet of transplantation is the development of a successful storage medium. We found tissue-culture methods most successful. The developing third molars of the 30–40-day-old male Syrian hamster were used as the potential transplants. After storage, these teeth were evaluated (1) by autogenous transplantation to a second molar socket and (2) by in vitro vital staining. The standard bone-bank tissue-culture techniques were modified and adapted to storage of dental tissues. Present clinical observations suggest that tissue culture in a medium of embryo extract, Tyrode's solution, and phenol red, plus penicillin and streptomycin, was most satisfactory. Seventy-five per cent of the autogenous transplants in this group were clinically firm and functional after 60 post-insertion days. Teeth stored as above and incubated in a vital stain—trypan blue—had granules of the dye within the cytoplasm of the phagocytic cells of the pulp. These observations suggest some maintenance of tissue vitality during the storage process.

29. ENAMEL ON CUSPS OF MANDIBULAR FIRST MOLARS IN ALBINO RATS.—*Leif B. Johannessen, Altadena, California.* The occlusal surface of the cusps of molar teeth in albino rats is always devoid of enamel, according to the literature. The present study was undertaken to investigate whether, prior to occlusal wear, enamel might be present on the summits of the cusps. Mandibular first molars were removed from 14 albino rats when the animals were 17 days old. Ground sections, 100 μ thick, were prepared after the teeth had been imbedded in epoxy resin. The occlusal surface of the mesial

cusp was partly covered by enamel in many of the specimens. The quantity and distribution of this enamel differed from one rat to the other, as well as between the left and right molars in the same animal. The centrodistal cusp was covered by enamel in some of the samples. No enamel was found on the occlusal surface of the centromesial cusp, as opposed to the distal cusp, which in all the samples had a uniform layer of enamel coverage. It was concluded that, prior to occlusal wear, the summits of the cusps of rat molar teeth are not always devoid of enamel.

30. Electron Microscope Study of Collagen Formation in the Dental Pulp.—*J. K. Avery and S. Han, University of Michigan School of Dentistry, Ann Arbor.* A series of young hamster molar pulps was fixed in either osmium tetroxide or potassium permanganate. Because of the dense gelatinous nature of the pulp, osmium tetroxide did not appear to diffuse through and fixed pulpal tissue to the same extent as did potassium permanganate. Pulp tissue fixed with potassium permanganate with veronal acetate buffer and calcium chloride revealed a clear picture of the structural elements of the pulp, such as blood vessels, lymphatics, and collagen fibers. In the area immediately surrounding the fibrocytes or fibroblasts of the pulp were many collagen fibers exhibiting the typical 640 A banding. At higher magnifications, young collagen fibers were observed in contact along the outer cell membrane of the fibroblasts. Intracytoplasmic filaments near the surface of these cells probably play a role in fibrogenesis. The collagen fibers of the pulp which later function in dentin matrix formation may thus arise from the syncytium of fibroblasts within the pulp.

31. The Influence of Age Variance on the Osteogenic Potential of Bone-Marrow Tissue.—*Melvin H. Amler and Percy L. Johnson, Guggenheim Institute of Dental Research, College of Dentistry, New York University.* A preliminary study has been made of the use of polyvinyl plastic sponge* to transfer bone-marrow tissue of the rat to the anterior eye chamber. It was found that marrow-cell infiltration of polyvinyl sponge in the femur bone of 2 weeks' duration (not long enough for spicule formation to take place, as indicated by control section) produced osteogenic fibers, spicules, and finally a compact bone after transfer as an autograft to the anterior eye chamber. When the sponge, however, was implanted in the narrow cavity of the femur bone for only 1 week and then transferred to the anterior eye chamber, bone formation did not take place, even when left in position up to 10 weeks. This appeared to indicate that the degree of maturity of bone-marrow tissue infiltrated into the sponge has some bearing on its osteogenic potential. Transferring polyvinyl sponge as an autograft to the anterior eye chamber following its implantation in the marrow cavity of the femur bone for 2 weeks did not appear to alter its osteogenic potential or rate of osteogenesis as compared with a control section of sponge left *in situ*. Washed polyvinyl plastic sponge was proved not to be a bone-inducting substance by itself when implanted in the anterior eye chamber, as a control, for 6 months' duration.

32. Age Changes in the Vascular and Nerve Supply to the Molar Teeth of Rats.—*Sol Bernick, Department of Anatomy, School of Medicine, University of Southern California, Los Angeles.* Rats varying in age from 6 weeks to 1 year were used to study the changes in the vascular and nerve supply to the molar teeth as a result of aging. The visualization of the vascular patterns to the molar teeth was accomplished by the India-ink perfusion method. After the perfusion period, the heads were fixed in an alcohol–acetic acid-formol solution. The specimens were then decalcified and prepared for nitrocellulose imbedding in the routine manner. The imbedded blocks of tissue were sectioned at 100–150 μ thicknesses, and the cut sections were cleared by the Spalteholz method and mounted. For the demonstration of the nerve distribution to the molar teeth, heads from rats of the same age groups as used above were prepared for sectioning in the same manner as described above. The cut sections

* Ivalon Surgical Sponge, Simonize Co., Chicago, Illinois.

were first treated with enzymatic hydrolysis (1 per cent pepsin), hydrated, and then followed by silver impregnation by Pearson's silver gelatin method. In the young animals the terminal vascular network was traced into the odontoblastic-predentin border, whereas in the old animals the blood plexuses were limited to the pulpo-odontoblastic border. In the young rats the nerve terminals were traced as far as the pulpo-odontoblastic region associated with the odontoblasts. On the other hand, the nerve fibers were observed entering the predentin in the molar teeth of old animals.

33. Formation of Osteoblasts, Odontoblasts, and Osteoclasts.—*A. M. Hunt and K. J. Paynter, Faculty of Dentistry, University of Toronto, Canada.* Fourteen 2-day-old guinea pigs were injected intraperitoneally with H^3 thymidine (1 μc/gm). The animals were sacrificed at intervals of from 15 minutes to 24 days. Autoradiographic techniques were used to examine the demineralized lower jaws, which had been sectioned mesiodistally, parallel to the long axis of the lower teeth. Periosteal cells at the lower border of the jaw, pulpal cells at the apices of the growing molars, and areas of bone resorption were studied to determine the location of cells that had incorporated H^3 thymidine in their chromatin material. At the early sacrifice intervals, osteogenic cells adjacent to the osteoblasts, which covered the surface of the actively growing bone, were labeled. The osteoblasts themselves were not labeled. At a later interval, the osteoblasts were labeled. After 4 days, radioactive osteocytes were imbedded beneath the bone surface, and no labeled osteogenic cells remained in the periosteum. Odontoblasts were not labeled at the early sacrifice times, but adjacent pulpal cells had taken up H^3 thymidine. At $1\frac{1}{2}$ hours, a strip of approximately 6–12 odontoblasts was labeled. None of the odontoblasts above or below these were labeled. Some adjacent pulpal cells were radioactive. Osteoclasts were not labeled until the second day. At the fourth day, 15 per cent of the osteoclasts were labeled, but only on one or two nuclei. It was concluded that osteoclasts are aggregations of cells which divided some days previously and that they do not divide by mitosis.

34. Mitotic Activity in Rat Molar Tooth Germs.—*K. J. Paynter and A. M. Hunt, Division of Dental Research, Faculty of Dentistry, University of Toronto.* Young rats were injected intraperitoneally with tritium-labeled thymidine at a dosage of 1 or 2 μc/gm of body weight in order to follow mitotic activity in molar tooth germs during development. The radioactive thymidine becomes incorporated in the nuclei of dividing cells, where it remains for a considerable time after mitosis and where it can be located by radioautography. In one series, animals were injected once—at 3 days of age—and were killed at various intervals following injection; others received 2 or 3 injections, 3 days apart, starting at 3 days of age, and were also killed at various times following the last injections. The time of sacrifice varied from a few minutes to several days. In another series, rats were given a single injection at 14 days of age and were killed at various postinjection intervals. Radioautographs were made of sections of the jaws containing the molar teeth. Little activity was evident 15 minutes following injections; after 1 hour, however, cells were clearly labeled. Many of the labeled cells were related to the development and growth of blood vessels, particularly in the pulp. Most early pulpal mitoses were confined to the area adjacent to the root sheath, with little activity elsewhere. A considerable number of dividing cells were found in the stellate reticulum and stratum intermedium of the enamel organ, even after the formation of the crown was well advanced. Many were also observed in the inner enamel epithelium at the base of fissures in the molar teeth after deposition of dentin and enamel was well advanced down the sides of the cusps.

35. Chemical Changes within Oral Mucosa Associated with Aging.—*Donald E. Flieder, St. Louis University School of Dentistry, St. Louis, Missouri.* Specimens of human oral mucosa weighing from 10 to 50 mg. were obtained from patients ranging from three to seventy-two years of age. The tissue samples were judged to be free of

any pathology determined entirely by clinical criteria. The specimens were fractionated and analyzed for phospholipids (PL), perchloric acid–precipitable protein-bound hexose (PBH), perchloric acid–precipitable protein nitrogen (PN), mucoprotein-bound hexose (MPH), mucoprotein nitrogen (MPN), collagenous-bound hexose (CBH), non-collagenous-bound hexose (NCBH), collagenous nitrogen (CN), and non-collagenous nitrogen (NCN). The following ratios were constructed: PL/PN, MPN/PN, MPH/MPN, PBH/PN, CBH/CN, NCBH/NCN, NCN/CN. There were no significant changes in the PL/PN, MPH/MPN, CBH/CN, and NCN/CN ratios with varying age. Significant decreases in the MPN/PN, PBH/PN, and NCBH/NCN ratios were observed with increasing age. It was concluded that a reduction of both free and protein-bound carbohydrate within oral mucosa is associated with advancing age.

36. Mast-Cell Determinations of Gingivae in Periodontitis.—*Clifton O. Dummett, Veterans Administration Hospital, Tuskegee, Alabama; T. E. Bolden, Seton Hall University, Jersey City; and S. Goldsberry, School Veterinary Medicine, Tuskegee Institute, Alabama.* An extension of studies involving tissue concentration of mast cells was undertaken to determine the number of mast cells in the gingivae of patients affected with periodontal disease. All the cases selected had a predetermined hopeless prognosis, insofar as the retention of teeth was concerned. Biopsies of the gingivae were made of twelve patients. Gingivae were taken mainly from the mandibular anterior, maxillary, and mandibular molar areas. The tissues were fixed (Smith and Atkinson, 1956) and sectioned serially at 6 μ. Two sections were stained with hematoxylin and eosin, two with toluidine blue, and one section from each specimen was unstained. In the twelve cases examined, no mast cells were evident. Dog omentum similarly treated contained large numbers of mast cells. Another set of ten cases was selected, and restaining of tissues revealed mast cells in nine and none in one.

37. Histological Study of Childrens' Gingivae.—*M. Silberkweit, R. L. Hayes, and T. H. Simpson, College of Dentistry, Howard University, Washington, D.C.* The cellular activity of the gingivae of children was studied. Specimens were obtained from 21 children—13 girls and 8 boys. Ages ranged from six through thirteen years. Gingival tissue was obtained in the bicuspid and deciduous molar areas. Tissue was fixed at once, sectioned, and stained for plainimeter measurements and histologic study. Cellular densities were computed from drawings and microscopic evaluation. No pattern of cellular density was found for these first cases. Where mitotic activity was greatest, however, the rete pegs and stratum spinosum were wider. Narrow rete pegs and thin epithelium did not usually show mitotic activity. In most cases the keratinous layer was thin, and there was usually evidence of parakeratosis. This did not seem to be related to either the age or the sex of the subject.

38. Significance of the Capillary Circulation of the Odontoblastic Layer.—*Y. R. Mohammed, Cairo University School of Dentistry, Cairo, Egypt, and G. Van Huysen and D. A. Boyd, School of Dentistry, Indiana University, Indianapolis.* The blood vessels of the odontoblastic layer and the layer of Weil are capillary in size and relatively infrequent in number. Operative exposure of these layers during cavity preparation is followed by little or no extravasation of blood. Therefore, unless there is a massive exposure involving the pulp proper and its more numerous and larger vessels, the exposure would be difficult to detect clinically with the unaided eye. Since a microscopic exposure would admit bacteria and plastic filling materials under packing pressures, undetected pulp injury could occur without visible hemorrhage. This may account for so-called idiopathic and anchoretic pulpites.

39. The Golgi Apparatus of Parotid Acinous Cells of the Mouse.—*Harold F. Parks and Erling Johansen, University of Rochester School of Medicine and Dentistry, Rochester, New York.* Thin sections of parotid glands of white mice were examined with the electron microscope. The Golgi apparatus was seen to consist of two kinds of

smooth-surfaced membranous components: closed flattened membranous sacs called *lamellae,* of variable length and width and about 200–300 A in thickness, usually arranged parallel to one another in compact groups; and spherical *vesicles,* 300–600 A in diameter, usually randomly distributed in the vicinity of lamellae. Golgi lamellae appeared to give rise to secretion granules by a process of enspherulation. Golgi vesicles, on the other hand, appeared to function as a medium of communication between endoplasmic reticulum and Golgi lamellae. Both the flat, rough-surfaced membranous cisternae of the endoplasmic reticulum and the Golgi lamellae presented profiles from which it could be inferred that they were either pinching off, or coalescing with, Golgi vesicles. Whether vesicles were passing from endoplasmic reticulum to Golgi lamellae, or vice-versa, or both cannot be stated with certainty at present. If the first-named possibility is correct, it seems likely that secretion antecedents are synthesized in the endoplasmic reticulum, transferred to the Golgi lamellae by vesicles, and stored in the lamellar membrane as secretion granules.

IV. NUTRITION. MARCH 23, AFTERNOON, SECTION D

40. The Effect of Metaphosphoric Acid in the Diet of Weanling Hamsters as Traced with P^{32}.—*J. Tuba, L. Heppler, and C. Castaldi, Faculty of Dentistry, University of Alberta.* Forty-two weanling hamsters were divided into 4 groups. One group was fed the basic cariogenic diet as suggested by Nizel and Harris. A second and third group were fed the same diet but with the phosphorus level increased 2 and 5 times, respectively. The fourth group served as controls. After 36 days the animals were injected intraperitoneally with P^{32} in the form of Na_2PO_4. Each received 0.5 ml. containing 50 mc. Counts were made of equal weights of spleen, liver, kidney, lung, heart, and equal volumes of blood from the experimental groups. Caries scores were made, and samples of whole teeth were analyzed for P^{32} uptake. Within 42 hours, about 85 per cent was excreted in the urine and about 2 per cent in the feces. P^{32} uptake in the soft tissues, blood, and teeth was the same for the 3 groups of animals. Metaphosphoric acid was most cariostatic for the groups receiving $\times 5$ the basic amount.

41. A Possible Role of Vitamin A in Sulfur Metabolism.—*James W. Smudski and Howard M. Myers, University of California School of Dentistry, San Francisco.* Among the clinical manifestations of the vitamin A deficiency state is the appearance of hyperkeratosis in various epithelia. Since this represents an accumulation of the protein keratin and since keratin is high in the high sulfur-containing amino acid cystine, the postulate has been made that vitamin A deficiency interferes with normal metabolism of sulfur. This could lead to an accumulation of the cystine as keratin in the epithelia of the body. To test this hypothesis, weanling male Long-Evans rats were divided into two groups, one fed a standard vitamin A–deficient diet with vitamin A added for normal growth and development, and the second fed the same standard vitamin A–deficient diet with no supplement. S35 metabolism was traced in both groups. The S35 activity related to total body weight was determined in the inorganic and ethereal sulfate fraction together and in the neutral fraction of both the blood and urine collected at 6-hour intervals following the IP injection of S35-labeled cystine. Data indicated that a slower rate of S35 disappearance occurred in the inorganic and ethereal fraction of the plasma in the vitamin A–deficient animals than in the same fraction of the blood of the normal control animals. This S35 was in the sulfate form, indicating that cystine was being metabolized to the sulfate level. Data also indicated a higher level of S35 activity in the inorganic and ethereal sulfate fraction of the urine of the control normal animals than in the same fraction of the urine of the vitamin A–deficient animals.

42. Acute Osteolathyrism in Young Guinea Pigs.—*Barnet M. Levy, University of Texas Dental Branch, Houston.* Young guinea pigs 4–6 days old were force-fed

5 mg. aminoacetonitrile three times daily. In 5–12 days all treated animals lost weight; their limbs were distorted; they had a weakness in the hind limbs; and large bony masses were seen or could be felt at the muscle attachments on the hind legs, forelimbs, and mandible. Histologic examination of the long bones, ribs, and condyles showed marked deformities of the cartilaginous growth zones and dramatic exostoses at the site of muscle attachment.

43. Effects of Antioxidants on the Caries Experience of Mice Fed a Purified Cariogenic Diet.—*Max Shapiro, Lasar Galpern, and B. H. Ershoff, Western Biological Laboratories, Culver City, California.* Male mice of the Webster strain were selected at 11–14 gm. in body weight and were divided into 4 comparable groups of 15 animals each. Group I was fed a highly purified diet consisting of cerelose, 59 per cent; vitamin-free test casein, 24 per cent; cottonseed oil, 10 per cent; Wesson modification of Osborne-Mendel salt mixture, 5 per cent; cellulose, 2 per cent; and the known vitamins. Group II, III, and IV were fed a similar diet, supplemented with 0.5 per cent DBH (2,5-di-tert-butyl-hydroquinone), DPPD (*N, N'*-diphenyl-*p*-phenylenediamine), and propyle gallate, respectively. Food and water provided ad libitum. Animals were sacrificed after 105 days of feeding, and each molar tooth was graded for caries on a scale of 0–5 (ranging from none to severe). One hundred per cent of the mice in all 4 groups exhibited caries. The percentages of carious teeth per mouse were: Group I, 20 per cent; Group II, 27 per cent; Group III, 25 per cent; and Group IV, 49 per cent. The average caries score per mouse was: Group I, 3.2; Group II, 4.6; Group III, 5.6; and Group IV, 9.7. It is apparent from these findings that propyl gallate at a 0.5 per cent level in the diet significantly increased the incidence and severity of caries in mice fed a highly purified cariogenic diet. Similar studies were conducted with mice fed the above antioxidants in conjunction with a natural food stock ration (Rockland Rat Diet in meal form). In contrast to findings on the purified diet, none of the mice fed the stock ration or the stock ration + 0.5 per cent DPPD developed caries. Four of 15 mice fed the stock ration + 0.5 per cent propyl gallate and 5 of 15 fed the stock ration + 0.5 per cent DBH had carious lesions, but the average caries score for animals in these groups was less than 0.75 per mouse.

44. The Effect of Milk-Powder Diets on Caries and Growth in Rats.—*Barden E. Patterson, University of Texas Dental Branch, Houston.* An attempt was made to produce smooth-surface lesions in the molars of cotton rats by feeding the animals a diet known to produce these lesions in white rat molars. The white rat controls developed these lesions; however, the cotton rats were completely resistant. A lysine supplement decreased smooth-surface lesions in the white rats, as expected. The cariogenicity of this diet was demonstrated by the presence of pit and fissure lesions in the cotton rat. The pit and fissure caries scores were determined, and the effect of autoclaving the skim-milk portion of the diet was studied. It was found that this procedure did not alter the cariogenicity of the diet and that the incidence and extent of pit and fissure type lesions in cotton rats were also decreased by the addition of lysine. It was found that the skim-milk-powder diets which promoted growth in white rats did not promote optimum growth in cotton rats, probably because it did not contain sufficient protein.

45. Lysine Deficiency Studies in Albino Rats.—*E. L. Smith, K. O. Madsen, and E. J. Edmonds, University of Texas Dental Branch, Houston.* McClure *et al.* reported increased smooth-surface caries and a severe growth depression in albino rats when the skim-milk powder of the cariogenic diet was autoclaved. Supplementation with *L*-lysine improved growth and decreased caries. Growth was greatly impaired by the autoclaved-milk diet even in the presence of added lysine. In the present experiment, the milk diets were prepared as McClure did. In our experiment the difference in response of males and females was great enough to require them to be treated separately. On every experimental diet the females had a higher average caries incidence

than did the males. However, this sex difference was statistically significant in only those rats fed the unautoclaved basal diet and again in the rats fed the autoclaved diet supplemented with lysine. Males fed unautoclaved basal diets, males fed unautoclaved basal diets supplemented with lysine, males fed autoclaved lysine-supplemented diets, females fed unautoclaved supplemented diets, females fed autoclaved, and females fed autoclaved and supplemented diets were indistinguishable statistically. Females on the unautoclaved diet had statistically more, and males on the autoclaved supplemented diet had significantly fewer, caries than did the other six groups. With every diet, males gained more weight than females, but within a sex only the autoclaved supplemented diet gave a statistically significant weight gain. Supplementing the autoclaved diet did return the calcium content of femur bone ash to nearly normal. Phosphorus changes in bone ash were not necessarily opposite to calcium changes.

46. Observations on Alveolar Bone Resorption in Rodents Fed a Diet Containing Ground Whole Oats.—*Paul N. Baer and F. J. McClure, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland.* In a previous study it was reported that a diet consisting predominantly of ground whole oats, when fed 90 days to weanling Sprague-Dawley rats, resulted in the extensive loss of alveolar bone. To extend this information, 70 Sprague-Dawley rats, 24 mice, and 24 hamsters were studied in more detail after being maintained on the whole-oat diet for periods of 30 days or more from weaning. In an additional 108 rats, two other diets were studied. In the one, whole-wheat flour and in the other ground rolled oats* was substituted for the whole ground oats. When gross defleshed specimens were examined, all 3 species which were fed the whole ground-oat diet for at least 30 days showed extensive loss of alveolar bone about the molar teeth. The bone loss was associated with the impaction of food and hair. With increase in age, the lesions became more severe. Histologically, in all three species, true periodontal pockets were present. In the rats maintained on the whole oat diet for 90 days, the sulcular epithelium had migrated apically beneath the molar roots, radicular abscesses were present, and the lingual plate of alveolar bone was absent; the buccal plate was intact and showed the presence of an osteophytic type of bone formation. The mice and hamsters showed a somewhat similar histologic picture, except that osteophytic bone was not observed. These effects did not occur in rats fed the diets containing whole-wheat flour or ground rolled oats.

47. Serum Thiamine Levels in Hamsters on a Thiamine-low Diet.—*J. R. Eshleman, J. J. Salley, and J. H. Morgan, Department of Oral Pathology, Medical College of Virginia, Richmond, Virginia.* A microanalytical technique for serum thiamine determination was developed to provide data relative to the minimum daily requirement for the survival of Syrian hamsters on a thiamine-low diet. This method of analysis allowed repeated individual sampling rather than the use of pooled samples or sacrifice of the animal. It represents modifications of the microbiologic-assay technique described in the Difco Manual for serum thiamine levels. Sample size was 0.5 cc. of blood obtained either by cardiopuncture or femoral vein cut-down. Samples of larger quantity could not be used without impairing the blood volume of the animal. The Difco technique requires samples containing a minimum of 0.25 μg. of thiamine, whereas some samples in this study contained as low as 0.002 μg. Twenty-three hamsters receiving a nutritionally adequate semisynthetic ration providing a daily intake of 46 μg. of thiamine HCl per hamster showed a range of 19.2–29.0 μg. thiamine/100 cc of blood, with an average value of 24.3 μg/100 cc. Twenty-nine animals receiving the same diet but with a daily thiamine intake of 23 μg/hamster demonstrated a range of 0–23.7 μg thiamine/100 cc blood, with an average value of 9.2 μg/100 cc. Hamsters receiving less than 23μg thiamine/day did not survive, thus establishing this figure as a reasonably accurate minimum daily requirement for survival in chronic thiamine-deficiency studies.

* Quaker.

48. Effect of Chronic Thiamine Deficiency on Oral Carcinogenesis.—*J. J. Salley, J. R. Eshleman, and J. H. Morgan, Department of Oral Pathology, Medical College of Virginia, Richmond, Virginia.* A total of 54 PeeDee strain albino hamsters was divided into 2 littermated groups. The experimental group of 32 was maintained on a semisynthetic ration containing a low level of thiamine (0.02 gm/5 kg. of ration). Control animals were fed the same diet but with a thiamine content of 0.04 gm/5 kg of ration. When signs of deficiency were evident in deficient hamsters, as noted by weight loss, all animals were topically treated in the right cheek pouch with 0.05 per cent 9,10-dimethyl-1,2-benzanthracene in an acetone vehicle 3 times per week. Results of this investigation showed that, although the incidence of induced tumors was the same in each group (100 per cent), the mean latent period for the deficient group of 27 survivors was 126.4 days, while this figure was 145.0 days in 22 animals receiving an adequate amount of thiamine. Subjection of these data to statistical analysis by the *t*-test revealed a probability of less than 0.01 that this difference was due to chance alone.

49. Effects of a Vitamin A–Deficient Diet on the Oral Mucosa of the Rabbit.—*C. Gigoux, J. Meyer, and J. P. Weinmann (deceased), University of Illinois, College of Dentistry, Department of Oral Pathology, Chicago.* The purpose of the investigation was to determine the epithelial changes associated with experimentally induced keratinization of oral epithelium. Twelve New Zealand White rabbits, aged 2 months at the beginning of the experiment, received a vitamin A–free synthetic diet (Lamming *et al., J. Nutrition,* **52**:217, 1954); 12 control animals were given the same diet with the addition of vitamin A. The experimental periods ranged from 12 weeks to 5½ months. Vitamin A levels were determined in serum and liver at the time the animals were killed. The serum vitamin A level in the 12-week experimental animal was found to be a fifth of that in the control partner, and the liver level about a third. Hyperkeratinization of the cornea was observable with the naked eye after about 2 months. Histologic sections were prepared by routine methods, as well as with the use of special staining techniques, as applied by Weinmann *et al.* (*J. Invest. Dermatol.,* **34**:423, 1960) to the distinction of different types of keratinization in oral epithelium. Epithelium with experimentally produced keratinization was studied in histologic sections of the posterior portion of the cheek, which is unkeratinized in the normal rabbit. This epithelium was compared with its unkeratinized counterpart in the control animals, on the one hand, and with normally keratinizing regions (palate), on the other hand.

50. Tooth Development in Deficiency Animals Receiving Vitamin D Supplements.—*B. R. Bhussry and Saroja Rao, School of Dentistry, Georgetown University, Washington, D.C.* Calcification disturbances in teeth of young rats whose mothers were fed vitamin D–deficient diets have already been reported. This investigation is concerned with the tooth development in the offspring of deficient mothers who received vitamin D supplements during pregnancy. Three groups of 12 female rats each were used in this study. The 2 experiment groups, depleted of their vitamin D reserves, received the Steenbock diet (Ca:P, 4:1), and the Chick Basal diet (Ca:P 1–2:1), respectively. The third group, used as controls, consisted of normal animals on stock diet. Animals from all groups were mated with Chick Basal males. Upon determination of pregnancy, the females were removed to individual maternity cages. Beginning at the fifth day of pregnancy, females in the experimental groups received a biweekly supplement of 50 units of vitamin D in cotton-seed oil by stomach tube. This supplement was continued after parturition until the weaning of the offspring was completed. As the litters were born, the young animals in each of the 3 groups were sacrificed at corresponding intervals (3, 6, 9, 12, 15, 18, 21 days). The jaws were fixed in neutral buffered formalin, decalcified, and processed by routine histologic procedures. The serial sections were stained with hematoxylin and eosin and Masson Tri-

chrome for microscopic examination. The observations suggest that the developing enamel demonstrated slight improvement in offspring from Steenbock group of animals receiving vitamin D supplements. The ameloblastic layer showed some recovery and produced a better quality of enamel matrix. The stratum intermedium, however, failed to reveal any significant change. Alterations in the odontoblastic layer and dentin were similar to those noted in the teeth of animals not receiving any vitamin D supplement. The calcification of enamel and dentin matrix, however, was not normal.

51. The Effect of Sodium Phytate and Sodium Acid Pyrophosphate on Rat Caries.—*S. Limbasuta, B. Singh, and E. Johansen, University of Rochester, Rochester, New York.* Male and female albino rats 26–27 days of age were littermated into one control and one experimental group for each sex. The control groups received the following diet: 35 per cent sucrose, 30 per cent whole powdered milk, 30 per cent whole-wheat flour, 4 per cent alfalfa, and 1 per cent sodium chloride. In the experimental groups, 2 per cent of the whole-wheat flour was replaced by an equal amount of sodium phytate or sodium acid pyrophosphate. The numbers of animals in each group at the termination of the study were as follows: male control, 18; male experimental–sodium phytate, 13; female control, 17; female experimental–sodium acid pyrophosphate, 15. The experimental period was 100 days. On the basis of the mean caries score the sodium phytate group showed no significant reduction in caries, while the sodium acid pyrophosphate group showed a statistically significant reduction.

52. Thyroid Activity and Dental Caries in Rats Fed a Powdered Diet.—*John Haldi, Winfrey Wynn, and M. L. Law, Emory University School of Dentistry, Atlanta, Georgia.* The experiments of Muhler and Shaffer indicate a relationship between the functional activity of the thyroid gland and susceptibility or resistance to dental caries. Inasmuch as their experiments were conducted on rats fed a coarse corn-particle diet with possible involvement of fracture of the tooth enamel, we have conducted somewhat similar experiments with a finely powdered high-sucrose diet. In one experiment albino rats were thyroidectomized and their control littermates subjected to a sham operation. The caries score of the thyroidectomized animals was 42 as against 29 for their controls. In a second experiment, triplicate littermates were fed, respectively, the control diet and the same diet containing either 0.05 per cent thiouracil or 0.1 per cent desiccated thyroid. The caries scores on these three diets were, respectively, 17, 25, and 10. It would appear from these two experiments that loss or reduction of the thyroid secretion to the body economy resulted in an increase in caries, whereas an increase in the thyroid hormone led to a decrease in caries. In a third experiment, triplicate littermates were sialoadenectomized and fed the same diets as in the second experiment. The caries scores were, respectively, 46, 44, and 41. It is therefore concluded that the influence of the thyroid gland on resistance to dental caries in rats fed a synthetic fine-particle diet was mediated via the salivary glands.

V. DENTAL CARIES I. MARCH 24, MORNING, SECTION A

53. Caries Susceptibility as Affected by Electrolyte Balance.—*Ralph R. Steinman, School of Dentistry, College of Medical Evangelists, Loma Linda, California.* Recent experiments have shown that stress applied to young rats significantly increased caries (*J. S. Cal. D. A.*, **28**:367, 1960). Prominent among various physiological changes induced by stress is a change in electrolyte balance. Since the water-transport system would be altered under these circumstances, the nutritive state of every cell in the body might be affected. This becomes significant when it is recalled that ligating arteries to teeth increased caries in rats (*J. D. Res.*, **37**:1069, 1958). A poor nutritional state may also be related to an increase in caries (*J. D. Res.*, **37**:865, 1958). It was therefore considered important to determine whether an altered electrolyte balance could be a factor in caries susceptibility. One hundred and eighty-two Osborne-Mendel rats were placed on a cariogenic diet (*Proc. Exptl. Biol. Med.*, **94**:391, 1957) at 21

days of age. At 31 days of age the teeth were studied histochemically (*J. D. Res.*, **38**:592, 1959) to determine the presence of incipient carious lesions. The following differences were observed: *Control,* 44 animals, water ad lib, score 7.00; 41 littermates, saline 0.9 per cent ad lib, score 10.44. *Control,* 26 animals, score 6.72; 30 littermates, Diuril 1 mg/l/gm/ration, score 3.68. *Control,* 21 animals, score 6.08; 20 littermates, DOCA (Percorten) 0.1 ml/35 gm/wt at 21 days of age, 0.05 m. at 28 days of age, score 9.88. Chi-square analysis of the data gives better than 1,000 to 1 that the observed difference was not due to chance. It would thus appear that an imbalance of the electrolytes could be one systemic factor affecting caries susceptibility under stress.

54. The Effects on in Vitro Caries of the Diffusion of Tryptophane, Niacin, Pantothenate, and Folic Acid through Human Teeth.—*L. R. Brown, Jr., Naval Dental School, National Naval Medical Center, Bethesda, Maryland.* The purposes of this study were to measure the rate of diffusion of different microbial growth factors through teeth and to evaluate the effects of this diffusion on the development of in vitro caries. Twenty extracted teeth (unerupted third molars) were mounted individually in plastic tubes designed to form an external seal between the crown and the root of the tooth. The crowns of the teeth were covered with distilled water, and the roots were immersed in separate solutions of tryptophane, niacin, pantothenate, and folic acid, or a solution containing a mixture of all four nutrients. Using standard microbiologic-assay techniques, the various nutrients diffusing through the teeth were measured by sampling the crown solutions at regular intervals. All nutrients diffused through the dentin at approximately the same rate: equivalent in volume to about 0.001 ml/day. When the crowns were covered with a culture medium deficient in one or all four nutrients and inoculated with *Lactobacillus arabinosus* or *Streptococcus faecalis,* the nutrients at the roots diffused through the dentin in sufficient amounts to support growth. After 12 weeks the resulting dentinal changes resembled natural caries, in that deep penetrating lesions with microbial invasion of the dentinal tubules were observed. These changes were not observed in teeth where the crowns were covered with a medium, deficient or complete in the nutrients, and the roots immersed in distilled water.

55. The Effects of Anticaries Agents on Artificially Induced Carious Lesions.—*Donald L. Opdyke, Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio.* By using the agar method of Francis and Meckel, modified by using lactic acid as the demineralizing agent instead of bacterially produced acid, it is possible to produce carious lesions in human teeth that are indistinguishable, morphologically or histochemically, from naturally occurring dental decay. By treatment with enamel antisolubility agents before, or concomitant with, the exposure in the agar system, this carious process can be prevented. Several agents have been compared for protective action. In addition, after incipient carious processes have been induced, treatment with stannous fluoride solution causes the lesions to lose all the distinguishing characteristic histochemical reactivity, and the lesions disappear. One method by which the former carious sites can be visualized is by specific staining for tin.

56. Influence of Kind of Acid, Molarity, pH, and Saturation on the Penetration of Enamel.—*K. K. Kapur, Eugene E. Fischer, and R. S. Manly, Tufts University School of Dental Medicine, Boston.* The edge decalcification technique was applied to a study of the importance of the kind of acid, its molarity, pH, and the degree of saturation on the rate of penetration in microns per day. The first study was concerned with three different acids at 1 molar concentration and six pH's ranging from 4.0 to 6.0. During the first 5 days the rate of penetration for lactate buffer was essentially linear and about seven times as great at pH 4 as at pH 6. A similar range held for acetate except that the penetration rate was from one-fourth to two-thirds

as great. Findings with citrate were less reproducible, presumably because of its tendency to produce cavitation rather than decalcification. Penetration rate decreased both with increase in pH and decrease in concentration, and the two variables interacted. At high pH, the first halving of concentration produced the greatest change, whereas at low pH the last halving of concentration produced the greatest change. In a study of four degrees of saturation at two different pH's and molarities of lactate buffer, the degree of saturation appeared to be least important.

57. Inhibitory Effect of Disodium Phosphate on Dental Caries in the Rat.—*A. E. Nizel, R. S. Harris, and J. M. Parker, Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Cambridge.* This experiment was designed to estimate the level at which Na_2HPO_4 can be fed in the diet of rats in order to give maximum caries resistance without interfering with growth and development. The control caries-producing diet contained 0.4 per cent P and was composed of corn 47, sucrose 14, non-fat milk solids 20, plastic cream 10, alfalfa meal 6, liver concentrate 2, and NaCl 1. The three test diets were prepared by adding 1.81, 3.62, and 5.43 per cent Na_2HPO_4 to the control diet at the expense of the four major components, thereby doubling, trebling, and quadrupling the P content. At 3 days of age the pups of 8 litters were interchanged between mothers, and groups of 16 pups each were placed on the diets 100 days. At autopsy the average caries scores of the four groups were 42.9, 14.8, 15.2, and 22.5, respectively. The groups which received double-P and treble-P diets showed normal food consumption and body-weight gain and showed 65 and 64 per cent reductions in caries scores in comparison with the controls. The group receiving the quadruple-P diet showed reduced food consumption, decreased growth, and erratic caries scores. Thus best results were observed with the double-P diet, for the treble-P diet was no more effective against caries and the quadruple-P diet did not promote satisfactory growth.

58. Morphologic Differences in Teeth of Caries-Susceptible and Caries-Immune Males.—*W. R. Shiller and R. B. Wolcott, Dental Research Facility, Great Lakes, Illinois.* This study was conducted to determine whether a difference existed in the dental morphology of caries-susceptible and caries-immune individuals. All subjects for the study were Caucasian male recruits. Their ages ranged from seventeen to twenty-two years. Fifty caries-susceptible and forty-nine caries-immune individuals were studied. The caries-immune group included only those men who had no caries experience, and the caries-susceptible group was composed of individuals with a DMFT score of 12 or above. The caries-susceptible group had significantly greater facial convexities of their maxillary second premolar and maxillary molar teeth. The caries-susceptible group had significantly greater cusp heights of all first premolars, of maxillary second premolars, and of mandibular first molars; and the susceptible group's teeth showed a greater reduction in the number of cusps than did the immunes. No definite explanations are offered for these differences.

59. The Effect of Anion-Exchange Resins on Experimental Caries.—*R. A. Giordano and J. H. Shaw, Harvard School of Dental Medicine, Boston.* Since anion-exchange resins have the capacity to neutralize acidic solutions, these insoluble compounds could provide a mechanism for rapid neutralization of free acids on tooth surfaces. A weakly basic* and a strongly basic† polyamine resin were selected for animal experimentation because of their acid-neutralizing capacities as determined by titration analysis. Littermates of Harvard caries-susceptible rats were distributed at weaning into eight groups of about 20 rats each and maintained for 60 days. Group 1 received cariogenic diet 700. Groups 2–5 were given dietary supplements of 5, 10, 20, and 40 per cent regenerated weakly basic resin,* respectively. Unregenerated strongly

* Amberlite XE-58.

† Dowex 2X8.

basic resin* was fed at 20 per cent to Group 6. The latter, used in Group 7 at the 20 per cent level, had been finely ground in a ballmill for 48 hours. Dowex 2X8 was used in Group 8 at 20 per cent. Significant reductions in growth rate occurred in rats receiving 20 and 40 per cent regenerated XE-58 and 20 per cent unregenerated XE-58. A 74 per cent reduction in average caries score was observed among rats fed 20 per cent regenerated XE-58 as compared with controls in Group 1. A 48 per cent reduction in caries score occurred with 20 per cent unregenerated XE-58, which has no acid-neutralizing ability, and a 75 per cent reduction with Dowex 2X8, which has an acid-neutralizing capacity one-fourth that of Amberlite XE-58. These reductions were significant at the 1 per cent level. Significant, but smaller, reductions in caries were observed in the other groups. These data suggest that other properties of the resins in addition to their acid-neutralizing properties were involved in these reductions in experimental caries.

60. Caries Activity in Rats in Relation to Source of Cariogenic Flora.—*Rachel H. Larson and Robert J. Fitzgerald, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Maryland.* A comparison was made of various methods of inoculating rats with cariogenic flora. In order to depress the normal flora, 0.25 per cent tetracycline was added to the drinking water of weanlings from the sixteenth to the nineteenth days of age. At 21 days of age all animals were placed on diet 580, and 3 groups of depressed animals were inoculated as follows: 7-week-old caries-active animals on diet 580 were either placed directly in the cage with the experimental animals for 4 weeks or used to provide cecum content, which was added, fresh or lyophylized, to the drinking water provided for 1 week. The average numbers of carious enamel areas on the buccolingual, proximal, and sulcal surfaces were as follows: controls—4.4, 1.7, 25.1; depressed animals—1.0, 1.2, 16.8; depressed plus lyophylized cecum content—1.8, 1.8, 20.2; depressed plus fresh cecum content—7.1, 5.0, 28.9; depressed plus caries-active rat—29.2, 11.5, 32.3. The major caries activity of the control animals occurred in the sulci. The animals pretreated with tetracycline and not inoculated showed a highly significant decrease in sulcal activity and some decrease on the buccolingual and proximal surfaces. The animals inoculated with lyophylized material showed only a slight increase in activity; those with fresh cecum content developed more caries activity on all surfaces than the untreated controls and significantly greater than the depressed controls. The animals housed with a caries-active animal developed the most extensive caries activity of any group, the greatest increases being on the buccolingual and proximal surfaces.

61. Dental Caries in Institutionalized Mongoloid Patients.—*Richard A. Winer and M. Michael Cohen, Tufts University School of Dental Medicine, Boston, Massachusetts.* In a recent report (Cohen, Winer, and Shklar, *J. D. Res.*, **39**:745, 1960) of a group of 100 institutionalized Mongoloid patients, ages one to thirty years, severe periodontal disease was found in 96 per cent. Clinical examination for caries in these patients showed a remarkably low incidence of carious lesions. The patients were divided into groups according to their chronologic age. Group I (ages one to six), 18 patients; Group II (ages seven to twelve), 30 patients; Group III (ages thirteen to seventeen), 25 patients; Group IV (ages seventeen to thirty), 25 patients. In Group I, no carious lesions were observed. Group II, mean DF teeth were 0.71; Group III, mean DF teeth were 1.84; Group IV, mean DF teeth were 3.84. Mean DF teeth for the entire group (100 patients) were 1.84. The mean DF teeth of the Mongoloid patients in Group III were compared with a group of mentally defective non-Mongoloid patients in the same institution. The mean DF teeth for the Mongoloid group were 1.84, and the mean DF teeth for the non-Mongoloid group were 3.55.

62. A Study in Experimental Human Dental Caries Test Design and Interpretation.—*David Bixler, Indiana University Medical Center, Indianapolis.* The

* Dowex 2X8.

experimental testing of possible therapeutic agents for anticaries effectiveness in the human is a process involving many variables. This report deals with a human clinical dental caries study designed to test the effect of treatments when using more than one examiner. Others have investigated the effect of more than one examiner on caries findings when all examiners looked at the same subjects; none of these studies used control and test treatment groups. In this study, test and control groups were involved, and the two examiners looked at a part of the subjects from each group. Both examiners employed the same methods for scoring caries and recording the results. In order to have the desired number of subjects, two military schools were selected with nearly identical environments. The children in each school were divided into two groups. Group I was given the non-fluoride dentifrice and Group II was given the stannous fluoride–containing dentifrice. Complete examinations for caries were performed in the same manner at the beginning of the test and after 8 months. The subjects using the stannous fluoride dentifrice had a significant reduction in the DMFT and DMFS increments. An analysis of variance applied to the data failed to show a significant interaction between the dentists and dentifrice. Thus, *in this study,* there was no significant difference in the ranking of the treatments by the examiners, even though DMF findings of each dentist were statistically different. One examiner judged larger DMF increments than did the other examiner.

63. Caries Incidence and Pulpal Pathology in a Strain of Chinese Hamsters.—*M. Michael Cohen and Gerald Shklar, Department of Oral Pathology, Tufts University School of Dental Medicine, and George Yerganian, Children's Cancer Research Foundation, Harvard Medical School, Boston.* Spontaneous hereditary diabetes mellitus in Chinese hamsters (*Cricetulus griseus*) has recently been described by Meier and Yerganian (*Proc. Soc. Exptl. Biol. and Med.,* **100**:810, 1959). This was of interest because an increased incidence of dental caries has been observed in humans with long-term diabetes mellitus (Cohen, *J.A.D.A.,* **34**:239, 1947). The diabetic hamster offered an experimental approach to the study of caries and pulpal pathology in diabetic animals. Twelve animals with spontaneous hereditary diabetes served as the basis for the study. Twelve normal animals were used as controls. All diabetic animals received insulin-replacement therapy. Using the dissecting microscope, it was apparent that there was a marked increase in incidence of caries in the experimental animals. Histologic examination revealed an increased depth of the carious lesions in the experimental animals. In addition to the carious lesions, notable pulpal changes were observed in both carious and non-carious teeth. In non-carious teeth there were degenerative changes in the odontoblasts and pulpal fibroblasts, as well as connective tissue degeneration. Many of the carious teeth showed severe inflammatory reactions, with pulpal abscesses and necrosis of pulp. Further studies are in progress.

64. Occurrence of Enamel Defects in Children with Allergies.—*Lewis J. Rattner, Merle E. Morris, and Howard Myers, University of California School of Dentistry, San Francisco.* A higher incidence of enamel defects in cerebral palsy cases as compared with the general population has been reported. This is consistent with other findings which suggest that many developmental aberrations or systemic disturbances are positively correlated with enamel defects. Severe allergic reactions which occur in the very young and continue into childhood have not yet been studied in this regard. Fifty children of both sexes between three and nine years of age were given full-mouth radiographic and clinical examinations. All the subjects had a history of an early-appearing allergy with general symptoms. Any defect in the formation of buccal or cuspal enamel was considered a positive finding. A reported incidence of 6 per cent enamel defects in the general population has been accepted as a control value for this study. At the 10 per cent level of confidence, 13 positive findings out of 44 cases would be significant. To date, 15 positive findings out of 28 cases have been made. The most prevalent lesions have been found in the deciduous first molars and cuspids.

65. SOFT X-RAY STUDIES OF CARIOUS DENTIN.—*Manawel Araiche, Erling Johansen, and Harold F. Parks, Departments of Dental Research and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, New York.* Electron-microscopic studies performed in our laboratory have revealed an irregularly distributed residue of apatite crystallites in soft carious dentin. Since individual sections studied in electron microscopy reveal the conditions in very limited areas, it was of interest to gain a more general picture of mineral distribution. For this purpose it was decided to study ground sections of carious dentin with soft X-rays. Observations were made on approximately 200 individual sections obtained from 13 molars with large occlusal carious lesions. The sections were cut with a diamond wheel and ground to a thickness of 90–100 μ. Microradiograms were recorded on high-resolution spectroscopic slides with a Picker industrial X-ray unit operated at 25 kv 10 ma for a period of 2–3 minutes. It was occasionally found that a superficial zone of a lesion was more highly mineralized than deeper parts. In most of the lesions, thin bands of hypermineralized material were seen running more or less transversely to the dentinal canals; these bands were continuous with similar formations in the adjacent sound dentin. Diffuse globular patches of hypermineralized matrix were also seen at various levels in the lesion. In most of the specimens studied, canalicular structure could be seen in all levels, including the most superficial parts of the lesion.

VI. MICROBIOLOGY I. MARCH 24, MORNING, SECTION B

66. RESURVEY OF MICROBIOLOGIC HAZARDS IN DENTAL PRACTICE.—*Doran D. Zinner, National Children's Cardiac Hospital, Miami, Florida.* In 1956, a state-wide survey of the Florida dentists in active practice was conducted, in order to ascertain the sterilization and injection techniques in common use for dental local anesthesia. The results disclosed some questionable methods of sterilizing techniques practiced in the average dental office, including 31.4 per cent reuse of cartridges from patient to patient. A national educational campaign, including editorials and articles emanating from the American Dental Association soon followed, accompanied by revisions of the armamentaria from dental manufacturers. In 1960 a follow-up survey of 1,680 dentists was initiated, covering a more comprehensive questionnaire. This survey, conducted by salesmen from a state dental-supply company, was done on a personal-interview basis. The questions included sterilization techniques for all major instruments and injectables utilized in dental offices, the school and year of graduation of each dentist, information on dentists previously infected with serum hepatitis, etc. The educational campaign on methods of sterilization of needles, syringes, and continuing reuse of cartridges has been very effective. The changes of the dentists and their awareness of sterilizing methods for instruments and handpieces is negligible. The rapidity with which they inject 2 cc. of anaesthetic solution may be the reason for the frequent side reactions experienced by many patients. This survey probably reflects dental practices that are prevalent in other areas throughout the country. Dentists practicing in this state received their dental education at all the schools in the United States and Canada. At present the percentage sampling is over 50 per cent and reports are still arriving.

67. TIME-TEMPERATURE RELATIONSHIP OF DENTAL INSTRUMENTS IN ROOT-CANAL INSTRUMENT STERILIZERS.—*Henry M. Koehler and John J. Hefferren, Division of Chemistry, American Dental Association, Chicago, Illinois.* Electrically heated, thermostatically controlled baths have been studied as devices for the sterilization of root-canal and other dental instruments. Glass beads, molten metal, and sodium chloride have been used as heat-transfer media in these baths. The temperature gradients of two baths containing the media were determined with copper-constantan thermocouples. Copper-constantan thermocouples were arc-welded to a spot convenient to the tip of root-canal and other dental instruments, so that the temperature of that part of the instrument could be determined subsequent to immersion in the heat-transfer media. The rate of temperature increase with time was automatically recorded after

the instruments had been immersed to known depths in the hot bath. The rise in temperature of the instrument at the point of thermocouple attachment was found to be a function of the depth of the instrument in the heat-transfer medium, the length of time of immersion, and the surface area of the instrument. For routine chair-side use, the time required for larger instruments to reach the recommended sterilizing temperature of 218° C. is longer than convenient. Where the heat-transfer medium used does not provide good heat conduction, many instruments did not reach the temperature of the bath.

68. Interruptions of Oral Ecology Related to Caries in the NMRI-D Rat. —*Carl A. Ostrom and Robert Van Reen, Naval Medical Research Institute, Bethesda, Maryland.* Using *Bacillus brevis* ATCC 8185 and 10027, attempts were made to establish a reported antagonist in the cariogenic flora: (1) weanlings on cariogenic diet 30P2 were seeded daily; (2) newborn pups were seeded daily until weaning; (3) weanlings on 30P2, with casein and fat substituted for the sugar, were seeded daily for 1 week, followed by diet 30P2; (4) weanlings were provided the cariogenic diet supplemented with 15 U/g bacitracin and 100 μg/g streptomycin and distilled water with the same antibiotic concentrations plus *B. brevis* spores. Weekly oral samples were cultured, and caries was scored after 35 postweaning days. The oral flora resembled normal in 1, 2, and 3, but in 4 the heterofermentive lactobacilli and 1 type of alpha streptococci were markedly reduced. Caries resembled normal in 1 and 2; in 3 the incidence and severity were reduced 10 and 30 per cent in the sugarless-diet control group, but the antagonist showed no further influence; and in 4 both incidence and severity were reduced about 30 per cent in the antibiotics control group, but the antagonist recovered from the oral samples. Since observed preweaning caries may have obscured preceding experiments, 2 supposedly non-cariogenic diets were fed through 2 generations: the cariogenic diet with casein and fat substituted for the sugar and Shaw's diet No. 170. The F 1 generations' altered growth patterns were associated with a bizarre caries picture, in that third molar caries incidence and severity were increased, while the second and first molar incidence was similar to normal and severity was reduced. Oral heterofermentive lactobacilli were appreciably reduced.

69. The Influence of Phosphates and Molybdate on Dental Caries in the NMRI-D Rat.—*Robert Van Reen and Carl A. Ostrom, Naval Medical Research Institute, Bethesda, Maryland.* A number of investigators have demonstrated a cariostatic effect of various phosphates when added to the diet of experimental animals. The present studies were designed to test the efficacy of dietary Na_2HPO_4, $(NH_4)_2HPO_4$, $Na_4P_2O_7$, and $Na_5P_3O_{10}$ in th weanling NMRI-D caries-susceptible strain of rat. The latter two compounds were tested by virtue of their acid-inhibiting potency as reported by others. Sodium phosphate at a level of 1.5 per cent was added to a basal purified type diet and the other compounds at 1.4, 1.4, and 1.3 per cent, respectively, to provide equivalent phosphorus levels. Caries severity scores after 5 weeks on experiment were: control, 37.2; Na_2HPO_4, 27.6; $(NH_4)_2HPO_4$, 27.2; $Na_4P_2O_7$, 36.4; and $Na_5P_3O_{10}$, 38.3. Using filtrates of these diets or Snyder's broth with the phosphates added, inoculation with oral sample from the NHRI-D rat and incubation at 37° for 24 hours resulted in all cultures with a pH of 4.8–5.2 and at 48 hours with a pH of 4.0–4.5. Literature reports have suggested that molybdenum may play a role in reducing dental decay. Studies were also conducted, therefore, to test this in the NMRI-D rat. Sodium molybdate was added to the basal diets at levels to provide 12, 24, and 48 ppm molybdenum. After 5 weeks on experiment, no reduction in severity scores was apparent. In a subsequent trial, weanling female rats were given 10 ppm molybdenum in their drinking water for several months. Pups from these mothers were divided into groups receiving 48 ppm molybdenum in the diet or the unsupplemented basal diet. Again no pronounced difference was observed in the caries severity scores of the two groups.

70. MINERALIZATION OF AN EXTRACT OBTAINED FROM FILAMENTOUS MICRO-ORGANISMS.—*J. Ennever and W. E. Snyder, Ivorydale Technical Center, Procter & Gamble Company, Cincinnati, Ohio.* This report covers the second phase of a study intended to provide a tool for investigating the fundamentals of biologic calcifications. Filamentous oral micro-organisms, previously shown to be capable of forming intracellular hydroxyapatite, were extracted with glacial acetic acid. The extract was reconstituted by dialysis against distilled water. The extract and the extracted cells were placed in calcifying solution for 1 hour. The treated extract was positive for calcium phosphate; the extracted cells were negative. The extract was suspended in calcifying solution at room temperature for 10 days. X-ray diffraction revealed that the extract was positive for hydroxyapatite. The extract was refluxed in 20 per cent hydrochloric acid, and the hydrolyzate was analyzed for amino acids. The following were detected: alanine, arginine, aspartic, glutamic, glycine, histidine (or serine), leucine (or isoleucine), lysine, methionine, phenylalanine, proline, tyrosine, and valine.

71. LOCAL AND SYSTEMIC EFFECTS OF ENDOTOXIN ADMINISTERED TO ORAL MUCOSA.—*Anthony A. Rizzo and Stephan E. Mergenhagen, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Maryland.* The marked susceptibility of oral mucosa to endotoxins from oral bacteria has been shown previously in experiments which demonstrated primary toxicity and the local Shwartzman reaction in the posterior gingivo-palatal area of the rabbit mouth. These investigations established that the lesions obtained were basically similar to skin lesions obtained with the same preparation. In the present work, a more purified lipopolysaccharide from the same *Veillonella* organism was isolated and tested further for biological effects. Prior treatment of acetone-dried cells with 0.5 per cent formalin prevented nucleic acid contamination of the extracted endotoxin, so that final dried material contained only 0.4 per cent nitrogen and 1.8 per cent protein. Varying amounts of this preparation were administered to rabbits either intracutaneously or intramucosally, and rectal temperatures were measured. Twelve hours after the local injection, intravenous doses were given to the same animals to test for the Shwartzman reaction in the local site and in the upper, deep, cervical lymph nodes. After time was allowed for lesions to develop, the animals were sacrificed and tissues prepared for histologic study. A single dose of as little as 1.0 μg. of endotoxin given intramucosally produced temperature elevation, whereas 25 times this dose given intracutaneously was not pyrogenic. The same intramucosal dose was adequate to prepare for the local Shwartzman reaction at both the injected site and the regional lymph node. The results of these experiments indicate that the release of very low levels of such bacterial products can have marked local and systemic effects.

72. A PREDETERMINED SELECTED ORAL FLORA MAY PREVENT CERTAIN COMMUNICABLE DISEASES.—*Charles A. Scrivener, College of Physicians and Surgeons, San Francisco, California.* Results of a clinical survey reported at the meeting of the I.A.D.R. in 1959 showed that a technique utilizing the inoculation of the bacterial antagonist *Bacillus brevis* into the oral cavity reduced dental caries by 50 per cent. Determination on the part of the investigators to produce an even greater reduction led to further animal experimentation. Seventeen strains of micro-organisms having antagonistic properties were selected from genera known to comprise the so-called "normal" oral flora. Viable cultures of these organisms were introduced into the mouths of caries-susceptible rats daily from birth for 100 days. A statistically significant reduction in caries of 56 per cent was found in the experimental group when compared with the control group. In the last days of the experiment the rat colony became infected with paratyphoid, and 70 per cent of the control animals died. None of the experimental animals showed ill effects. Two of the antagonists, *S. rimosus* and *B. polymyxa*, used in the oral inoculum were found to be antagonistic to the partyphoid group of organisms. Because it is known that the micro-organisms causing 90 per cent of man's com-

municable diseases enter the body system through the mouth or nose, it was thought possible that the selected induced oral flora might have intercepted the infecting organisms at the mouth level, thereby providing protection for the experimental animals. A group of protective organisms compatible with each other and with the mouth host has been selected, and a list of communicable diseases against which they may provide protection is prepared. Animal experimentation is being pursued.

73. Continued Study of Nocardia.—*Genevieve D. Roth, School of Dentistry, University of Kansas City.* A group of filamentous organisms was isolated from carious teeth on a semiselective medium containing neopeptone, gelatin, and bromcresol-green. A special technique was developed to secure smears. Oil-immersion studies were made directly on colonies on the agar plates, and, by using glass contact slides, it was possible to demonstrate the transitory filamentous or mycelial stage. Slight changes in the amount of the inoculum or in the constituents of the media caused variations in the metabolic by-products. This phenomenon leads to many false positives and may account in part for the confusion in published studies of these organisms. They possess certain characteristics of several closely related group of organisms (Skerman, *Genera of Bacteria*), *Propionibacterium, Micobacterium, Mycoccus, Cellulomas, Arthrobacter,* and *Corynebacterium.* A group of 28 has been thoroughly studied and classified as *Nocardia.*

74. Polysaccharide Storage by the Oral Microbiota.—*Ronald J. Gibbons, Sigmund S. Socransky, and John B. Macdonald, Forsyth Dental Infirmary, Harvard School of Dental Medicine, Boston.* Intracellular polysaccharides of the glycogen-amylopectin type are formed by a large number of micro-organisms and are believed to function as energy reserves, being formed under conditions where exogenous carbohydrate is plentiful, and utilized when the environmental carbohydrate is depleted. It seemed of interest to determine whether members of the oral microbiota were capable or not of such polysaccharide synthesis, because it could play a role in the microbial ecology of the oral cavity, as well as be of significance in the etiology of dental caries. Preliminary studies indicate that plaques suspended in buffer and incubated anaerobically with 0.6 per cent glucose for 1½ hours store from 15 to 25 per cent of the carbohydrate metabolized. To determine the proportion of the microbiota responsible, plaques were plated on trypticase soy agar containing 3 per cent glucose and incubated anaerobically for 3 days. The plates were then flooded with dilute iodine solution, and it was found that from 20 to 65 per cent of the resulting colonies gave staining reactions indicative of glycogen or amylopectin storage. Some of the organisms responsible have been shown to be streptococci, diphtheroids, and *Fusobacterium* spp. In addition, cultures of certain oral streptococci have been demonstrated to metabolize stored polysaccharides with the production of lactic acid. Plaque from caries-active and caries-free individuals was dispersed and plated on trypticase soy 3 per cent glucose agar. The proportion of colonies staining deeply with iodine was determined. In 12 caries-active patients the mean per cent of stained colonies was 55. For 9 caries-free patients the mean per cent stained was 30. The difference was highly significant.

75. Oral Microbiota in Relation to Oral and Systemic Disease.—*Robert M. Stephan, Evelyn Getz, Charlotte Daly, Arden Howell, Frances Paul, Rosemary Lederer, and Warren Ashe, National Institute of Dental Research, National Institutes of Health, U.S. Public Health Service, Department of Health, Education, and Welfare, Bethesda, Maryland.* This study is an attempt to explore certain aspects of the total oral microbiota and oral environment in patients admitted to the Clinical Center, N.I.H., for the study of rampant caries, advanced periodontal disease, and other oral and systemic diseases. All patients had complete oral and physical examinations. The particular methods employed have been (1) direct stereomicroscopic examination of the oral cavity at 6×–40× magnification, using selective stains, pH indicators, and microelectrodes to indicate reactions in localized areas; (2) phase-microscopic examination

at 1,500× magnification of samples of material taken from these localized areas and dispersed in special medium on a microculture slide; (3) Gram-stained smears and recently fluorescent antibody staining for differentiating organisms; and (4) cultures for a limited number of micro-organisms on selective media, including blood-agar and tomato-agar plates for bacteria, Sabourauds medium for fungi, egg-medium slants for amoebae and trichomonads, and tissue cultures for viruses.

Under the stereomicroscope, dental plaque material was visualized as intermixed colonies of micro-organisms of different colors and consistencies. Phase-microscopic examination showed many different varieties of micro-organisms and cellular debris. In material from carious areas, quantitative estimates showed that non-motile organisms predominated, usually cocci, yeasts, filaments, and short rod forms. In material from periodontal lesions there were usually a large number of leukocytes, and motile micro-organisms often predominated, including spirochetes, vibrios, trichomonads, and amoebae. No viruses were recovered from oral samples, although herpes virus was recovered in two cases from lesions on the lips.

76. The Relationship of Lactobacilli to the Presence or Absence of Teeth and Carious Lesions.—*Patricia Parsons and Philip Jay, School of Dentistry, Washington University, St. Louis, Missouri.* The purpose of this study was to evaluate the relationship of the presence of lactobacilli to the development of the dentition. Samples of saliva were collected from the mouths of newborn infants, infants, preschool children, and adults. The samples were cultured in dextrose-meat-infusion broth, pH 5.0, and plated on tomato-agar plates. The samples were examined for the presence or absence of lactobacilli, cocci, and yeasts. Lactobacilli were present in the mouths of 7 per cent of the 131 newborn infants from one to eight days of age. The frequency of occurrence of lactobacilli in the mouths of 640 infants in various stages of dental development of the primary dentition was between 27 and 35 per cent. Lactobacilli were present in the mouths of 88 per cent of the 190 children with complete primary dentitions, mixed dentitions, and permanent dentitions. No clear correlation could be determined between the counts of lactobacilli and clinical findings on examination on an individual basis. The counts of lactobacilli rose and fell with the presence or absence of natural or artificial teeth in the 13 patients examined before and after the placement of complete upper and lower dentures.

77. The Effect of Enamel Fluoride on Acid Production by *Lactobacillus casei*.—*William W. Briner, Proctor & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio.* The anticaries activity of fluoride treatments has been variously ascribed to antisolubility, antibacterial, and antienzyme effects. Jenkins, for one, found that the addition of as little as 2 ppm fluoride inhibited acid production by bacteria in a saliva-glucose mixture. The results of Jenkins were confirmed and extended to pure cultures of *L. casei* using NaF, SnF_2, and CaF_2 as the sources of fluoride. Experiments were conducted to determine whether fluoride from mottled teeth of high fluoride content or from teeth topically treated with solutions of NaF and SnF_2 was also available to decrease acid production. These teeth and untreated teeth were compared by exposing them to metabolizing suspensions of *L. casei* in micro-incubation cells, where the ratio of enamel surface to medium volume is large (12.5 mm^2/0.05 ml). Conversion of glucose to lactic acid by the bacterial suspensions was markedly inhibited in the presence of enamels with a history of fluoride exposure. A concomitant reduction in phosphate release was observed, which is explained by a combination of reduced solubility and lower acid production. Topical treatment of enamel with a high concentration of fluoride or the presence of fluoride in drinking water during tooth development caused incorporation of fluoride into the tooth structure. This fluoride was available to inhibit acid production by *L. casei*. These results suggest that an antienzyme mechanism may be responsible, in part, for the caries reductions observed following water fluoridation or the topical application of fluorides.

78. Fluorescent Antibody Typing of Hyaluronidase-producing Oral Enterococci.—*Burton Rosan, School of Dentistry, University of Pennsylvania, Philadelphia.* Subgingival scrapings from patients with periodontal disease were analyzed bacteriologically for strains which produced hyaluronidases. Each strain was tested for production by cultivation in a medium containing hyaluronic acid. It was found that this method of seeking the presence or absence of hyaluronidase activity was much more successful than analyses of culture filtrates or supernatants. The principal hyaluronidase producers were found to be *Streptococcus faecalis*. These strains, therefore, were studied in greater detail, to determine whether there were other properties or activities which might be associated with and thus indicative of hyaluronidase production. The standard biochemical reactions and environmental tests failed to indicate any dependable separation. Serological studies, using the indirect fluorescent antibody technique, suggested that rabbit antisera prepared with hyaluronidase strains were strain-specific. Reciprocal absorptions confirmed the specificity of each type of antiserum. Current work has been planned to test the usefulness of the antisera in separating many other strains newly isolated from patients with periodontal disease. If these relationships are supported, further work will be concerned with applying the technique to detection of such bacteria in tissue biopsies from periodontal patients.

VII. ELECTRON MICROSCOPY. MARCH 24, MORNING, SECTION C

79. Fine Structure of Cross-Striations in Prisms of Human Enamel.—*J. G. Helmcke, L. Schulz, and D. B. Scott, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Maryland.* Surfaces of non-carious human enamel, prepared either by fracture or by grinding, polishing, and etching, were examined by light microscopy (incident, transmitted, and phase-contrast), and regions were identified in which prism striation was evident. Series of shadowed positive carbon replicas of these selected areas were made for electron microscopy. The sequential technique provided two types of preparation, for the early films took the form of pseudo-replicas, actually bearing entrapped layers of crystalline and organic material, while the later films were true replicas. The cross-striations observed optically in the specimens themselves were recognized in both types of replicas, and a combination of structural features seemed responsible for the configuration. Gentle constrictions were noted along the prisms at the same regular intervals as those assumed by striations. In addition, certain characteristic arrangements of the ultra-structural components were seen within prisms. In the central prism core the crystals and organic elements were aligned fairly parallel to the prism axis, while in the cortical region they tended to incline outward, angulation being greatest in the widest portions of the prisms. As previously observed, the structures between the prisms resembled those within the prisms and were oriented obliquely between them. Since quantitative differences in the composition of prisms at regions of cross-striation were not evident, it was thought that the variations in structural organization must be responsible for the observed optical effects.

80. Ultra-Structure of Developing Human Enamel Electron Microscopy of Human Amelogenesis.—*E. Rönnholm,* Karolinska Institutet, Stockholm, Sweden, University of California at Los Angeles, and Zoölogy Department, California.* Human amelogenesis has been studied in 16–20-week-old fetuses by means of electron microscopy. Undecalcified embryonic tooth tissue was fixed either in osmium tetroxide solutions or in absolute alcohol. Decalcification was performed on ultra-thin sections, and section staining was applied to study the matrix. Observations have covered the stratum intermedium, the ameloblasts, the dentin-enamel junction. The analysis has been concentrated on the earliest stages in the development of enamel crystallites. These crystallites are formed extra-cellularly, and the striking feature is the rapid growth of the crystallites as judged from the large size of the first crystallites formed.

* Introduced by Dr. Sognnaes, University of California at Los Angeles.

The enamel crystallites are long, thin plates that aggregate to form long ribbons of crystallites. At the very earliest stages of enamel formation and particularly before the first enamel crystallites have occurred, collagen filaments have been observed extending from the dentin surface toward the plasma membrane of the ameloblasts. The collagen filaments are located between thin processes of the plasma membrane of the free surface of the ameloblasts. The first enamel crystallites appear to be formed between the collagen filaments. Collagen filaments have been observed only during the very earliest stages of amelogenesis. With this exception, the matrix of the enamel does not contain any collagen structures. It consists of thin filaments showing a high degree of orientation coinciding with the orientation of the crystallites. Electron-diffraction analysis has shown that the enamel crystallites are of the apatite type and are well oriented in relation to the underlying enamel matrix at the very first stage of development.

81. Effect of Heat on Collagen Fibril Structure in Lathyritic Rats.—*R. R. Bruns and M. L. Watson, University of Rochester, Rochester, New York.* Skin of normal and lathyritic rats was exposed to various amounts of heat and examined by light and electron microscopy. The homogenous, eosinophilic appearance of the dermis in lathyritic rats reported previously was found by electron microscopy to be associated with the presence of numerous fine filaments, which appear to be derived from degraded collagen fibrils. The latter are also found in normal rat skin, but only after exposure to greater amounts of heat. Thus it appears that collagen fibrils in lathyritic skin are substantially more sensitive to degradation by heat than are fibrils in normal skin. Fibrils in various stages of disorganization were observed, which suggest that the collagen fibril is degraded in the following sequence: (1) slight separation of filaments first evident in the interband regions; (2) further separation and distinct helical arrangement of filaments; and (3) complete disorganization of filaments. The relationship of these alterations to lathyrism will be discussed.

82. The Ultra-Structure of Hamster Cheek Pouch and Foot-Pad Epithelium.—*Max Listgarten and John Albright, Harvard School of Dental Medicine, Cambridge.* Hamster cheek pouch epithelium as visualized by optical microscopy is of the stratified, squamous, keratinizing type, 4–6 cells thick, in which intrinsic salivary glands and conventional rete pegs are conspicuously absent. Electron microscopy reveals a double-layered basement membrane separating the basal layer from the underlying reticular fibers of the dermis. Mitochondria are most frequent in the basal layer, though relatively few in number and not well developed. No definite Golgi network is seen. Desmosomes are relatively scarce in the basal layer but appear to increase in number nearer to the surface. In the superficial portion of the stratum granulosum their intercellular width increases, and an additional layer becomes interposed between the attachment plates. Relatively round keratohyalin granules appear in this layer. They show a dense internal honeycombed structure and are darker staining than the keratinized layer. In the stratum corneum they appear to become incorporated in the homogenous mass of keratinized cellular contents. A preliminary comparison with hamster foot pad reveals the following differences in the foot-pad epithelium: (1) the mitochondria are relatively fewer in number; (2) desmosomes are relatively more numerous; (3) keratohyalin granules have a more stellate, less rounded outline; and (4) the stratum corneum is thicker and denser.

83. Electron-microscopic Studies of Tooth Development in Rachitic Rats. —*Marie U. Nylen, National Institute of Dental Research, Bethesda, Maryland.* Previous ultra-structural studies of normally developing rodent incisors have shown that the intracellular components of the ameloblasts and odontoblasts become more abundant and highly organized as differentiation progresses. In addition, definite relationships have been established between cell differentiation, matrix formation, and mineralization. With these studes as background, it was decided to investigate with the electron microscope abnormalities in odontogenesis observed earlier optically in rachit-

ic animals. Female albino rats were maintained on a rachitogenic diet (Ca:P ratio = 4–5:1), from 3 weeks prior to mating. The offspring from these animals were sacrificed at 4 and 8 days. The mandibular incisors were fixed in osmium tetroxide and imbedded in methacrylate, and thin sections were cut from the apical toward the incisal end. Differentiation of both odontoblasts and ameloblasts, formation of the first layers of enamel and dentin matrixes, and initial mineralization of these first-formed layers seemed to take place in a normal manner. Past this stage there was a definite change in the pattern of dentin formation in the rachitic animals. Although there was an increase in the total thickness of the dentin-predentin layer, the rate of mineralization appeared reduced, so that the predentin layer was wider than normal. This change was associated with a change in the odontoblasts, which contained numerous vacuoles and large cisternae filled with a finely granular matter. Some variations were noted in the formation of enamel matrix, but their precise nature will require further studies.

84. An Electron-microscopic Study of Advanced Caries in Enamel and Dentin.—*A. C. W. Hutchinson, Lilia McMillan, and L. S. Fosdick, Dental School, Northwestern University, Chicago.* Teeth with macroscopic evidence of dental caries were selected. Attempts to replicate the lesions were unsuccessful except at the periphery. The softened enamel in the center was removed, stained with osmic acid, imbedded in acrylic resin, and sectioned. Other specimens of the softened enamel were homogenized, shadowed with chromium, and viewed. Much difficulty was experienced in obtaining adequate replicas of the dentino-enamel junction along which the earlier stages of caries had progressed. Here the lesion was observed as it spread both laterally and toward the pulp. Advanced carious dentin was treated similarly. It was found that the softened enamel consisted of crystalline material, in the main needle-shaped (possibly dicalcium phosphate dihydrate). It was heavily contaminated with micro-organisms. The dentinal tubule of advanced caries of dentin was packed with micro-organisms, surrounded by a thick network of organic material.

85. Fine Structure of the Trigeminal Ganglion.—*Andrew D. Dixon, Department of Anatomy, University of Iowa, Iowa City.* An electron-microscopic investigation of the normal structure of the trigeminal ganglion was undertaken as an essential prelude to a study of submicroscopic changes within such ganglia following lesions of dental nerve pathways. Trigeminal ganglia, removed rapidly from anaesthetized rats whose ages ranged from 3 to 93 days, were fixed in buffered osmium tetroxide and imbedded in an epoxy resin.* Ultra-thin sections were examined in an electron microscope,† and, where possible, morphological details were correlated with those observed in control tissue prepared for light microscopy by routine methods. Particular attention was given to the ultra-structure of nerve-cell bodies, their myelinated processes, and the unmyelinated component of the ganglion. The neuroplasm of the nerve cells was abundant in mitochondria and small electron-dense granules associated with either lamellar structures (to form Nissl bodies) or elongated vesicles. Distinctly lamellar myelin sheaths were observed around the axonal processes of the nerve cells, and the degree of myelin formation at different ages suggested that the process of myelinization continues for some time after birth. A noteworthy observation was the frequent occurrence within a single Schwann cell of more than one myelinated nerve fiber. Unmyelinated nerve fibers were grouped in small bundles, each fiber being contained in an invagination of a common, centrally placed Schwann cell whose scanty cytoplasm exhibited mitochondria and dense granules comparable with those seen within nerve-cell bodies.

86. An Electron Microscope Study of Invertebrate Cartilage Tissue.—*Philip Person, Delbert E. Philpot, and Ethel Suben, Veterans Administration Hospital, Brooklyn, New York, and the Marine Biological Laboratory, Woods Hole, Massachusetts.* Certain invertebrate animals possess true, mesenchymally derived endoskeletons

* Araldite, Ciba Products Corp., Fairlawn, New Jersey. † RCA EMU 3F.

of cartilage and cartilage-like tissues, which are of great importance for our understanding of the morphologic and biochemical evolution of skeletal and calcified structures. This report deals with the branchial cartilage of young specimens (2 inches long) of the horseshoe crab, *Limulus polyphemus*. This cartilage, in the electron microscope, reveals a unique matrix structure hitherto unreported. In the light microscope, the cartilage of such animals consists of small, closely packed cells, with deeply basophilic nuclei. The cells are surrounded by a very thin eosinophilic matrix. The cytoplasm of osmic acid–fixed material, when viewed in the electron microscope, contains typical components, such as mitochondria, Golgi apparatus, and endoplasmic reticulum. The matrix, however, is especially noteworthy and differs markedly from the matrices seen in vertebrate cartilages. As matrix first appears, it is a thin seam of amorphous material which separates adjoining cells. Later, on either side of this amorphous material, chains of rodlike micelles are formed in a lamellar, appositional fashion. These rods seem to take origin as elements of the endoplasmic reticulum and other cytoplasmic components, which, as they approach the periphery of the cell, give the appearance of being incorporated into the matrix. The lamellae form concentric layers. There are also fibrous elements, apparently lacking periodicity, in the matrix.

87. A Study of the Ultra-Structure Sequence in Healing Wounds.—*Russell Ross and Earl P. Benditt, Department of Pathology, University of Washington School of Medicine, Seattle, Washington.* A study of the regular sequence encountered in healing skin wounds was made with the electron microscope. Cell populations, their fine structure, and fibril formation in the connective tissues were observed. Linear incisions in the skin of normal male guinea pigs weighing 225–250 gm. were made. The wounds were examined at time intervals varying from 16 hours to 14 days after wounding. Tissues were fixed in cold 2 per cent osmium tetroxide buffered with *s*-collidine (pH 7.4) for 1 hour, post-fixed in cold, neutral buffered, formalin for 1 hour, and imbedded in an epoxy resin.

In the early phase (16–24 hours) the wounds were characterized by the presence of polymorphonuclear leukocytes and macrophages. Extra-cellular particulate matter seen resembled the cytoplasmic organelles of the neutrophiles. By 24 hours, less particulate matter was seen extra-cellularly, while large amounts were found within phagocytic cells. The first fibroblasts were identified at that time. The middle phase (3–7 days) consisted of large numbers of fibroblasts and early collagen formation. Fibroblasts were characterized by extensive development and dilatation of the endoplasmic reticulum, enlargement of their mitochondria, and numerous fine intracytoplasmic filaments. A few intracytoplasmic, electron-dense bodies were seen in scattered fibroblasts, and others showed occasional myeloid figures. In the late phase (9–14 days) the fibroblasts retained the same morphologic characteristics, the wounds decreased in cellularity, and the amount of extra-cellular collagen was increased. The mechanism of collagen fiber formation in relation to the cells will be discussed.

88. An Electron-microscopic Study of Dental Calculus.—*L. McMillan, A. C. W. Hutchinson, and L. S. Fosdick, Dental School, Northwestern University, Chicago.* Dental calculus, both old and freshly deposited, was obtained and studied by two distinct methods. Samples were fixed with osmic acid, imbedded in acrylic resin, and sectioned. Sections were cut parallel to the tooth surface and at right angles. Portions of the same specimen were homogenized and shadowed with chromium. The samples by both methods were then viewed in the electron microscope. All sectioned specimens showed micro-organisms and organic debris imbedded in a continuous phase of inorganic material. The homogenized specimens fractured in the inorganic phase and along the surface of the threadlike micro-organisms but never through microbial body. Freshly deposited calculus differed from the old calculus, in that the portions adhering to the micro-organism were more globular in form than those in the older specimens. It is suggested that the initial phase of calculus formation involves the deposit of calcium phosphate on or in the surface of threadlike organisms.

89. Electron-microscopic Surface Changes in Lacunar Resorption.—*Reidar F. Sognnaes, UCLA School of Dentistry, Los Angeles; John T. Albright, Harvard School of Dental Medicine, Boston; Robert M. Frank, University of Strasbourg, Institut Dentaire.* Small segments of the dentinal resorption surfaces of shedding deciduous teeth were imbedded in methacrylate, sectioned by means of a diamond knife mounted on a microtome,* and examined in an electron microscope.† The scalloped edges between individual Howship's resorption lacunae could be visualized at lower magnification. Where the section direction had produced centrally located profiles, it was discovered that the scalloped surface area exhibited a peripheral zone of about 0.6 μ, within which the hard tissue had undergone a distinct ultra-structural alteration. This change involved a reduced electron density due to an incipient surface loss of inorganic salts. This surface zone showed denuded fibrillar material and stood in sharp contrast to the intact hard-tissue matrix, which was extremely electron-dense. A priori one would suspect that bone and dentin are considerably more permeable than enamel and that the elements producing mineral dissolution in resorption would diffuse deeply into the subsurface zone. The fact that this does not occur or that it occurs only to a depth of half a micron, as compared with some 1,000 μ in enamel caries (R. F. Sognnaes, *Arch. Oral Biol.*, **1**:106, 1959), may indicate that the resorption process is intimately related to a virtually simultaneous mechanism capable of removing organic surface matter. Thus it may be suggested that, whereas in caries a primary inhibitory mechanism is most likely to be found in agents which prevent demineralization, the resorption mechanism would seem equally likely to be arrested by agents which would inhibit the enzymatic destruction of cellular and other organic elements situated along the surface of calcified tissues.

90. The Ultra-Histology of Bone Formation.—*James A. Yaeger, Department of Histology, College of Dentistry, University of Illinois, Chicago.* Mineralized and demineralized thin sections of developing bone from the palates of 1-day-old mice were examined in the electron microscope. The differentiation of bone from mesenchyme in areas of endomembranous bone formation can be described as occuring in 4 phases: (1) the appearance of collagen fibrils; (2) the aggregation of fibrils into fibril bundles; (3) the condensation of ground substance between fibril bundles and the simultaneous appearance of isometric inorganic particles; and (4) the aggregation of isometric inorganic particles into rodlets. The formation of bone by apposition upon pre-existing bone surfaces occurs in nearly the same sequence of phases. They are compressed into a relatively narrow zone, and isometric inorganic particles are not seen in all areas of appositional bone formation. An additional phase should be added, in which the collagen fibril bundles become more closely packed and the rodlets come to emphasize the collagen periodicity.

91. The in Vitro Effect of Salt Solutions on the Isolated Dental Pulp.—*Alben Curtis and M. B. Quigley, University of Alabama Medical Center, Birmingham.* Cross-banded collagen could not be found in electron microscope studies of the pulp. Instead, it was thought that tropocollagen was present similar to that which has been produced and isolated from rat-tail tendon and reconstituted to natural collagen with sodium chloride solutions. Unerupted teeth of young dogs were extracted and the pulps immediately removed from the pulp chambers. Some were used as controls and fixed for routine histologic study, while others were placed in 1, 2, or 5 per cent sodium chloride solutions for periods ranging from 4 to 24 hours, and then prepared for histologic examination in the same manner as the controls. The controls showed thin-walled vessels and poorly staining intercellular substance, which could not be definitely identified histologically as collagen. The salt-treated pulps, however, showed a marked perivascular, well-demarcated zone around the majority of the blood vessels. The background seemed to stain more acidophilic. The salt solutions did not affect the

* Servall-Porter-Blum microtome. † RCA model type EMU 3B.

cells as badly as expected. The perivascular "cuff" proved to be intensely blue with Masson's stain and to be markedly PAS-positive. In some instances the perivascular area appeared fibrous, while in others it seemed homogeneous. It was also noted that the intercellular material of the experimental group seemed more fibrous, but this was not as constant as the perivascular changes. Some of this material is now being prepared for electron microscopy.

VIII. E. H. HATTON AWARD COMPETITION I. MARCH 24, MORNING, SECTION D

92. THE BEHAVIOR OF THE PRESCHOOL CHILD AT THE INITIAL DENTAL VISIT.—*Martin Rosengarten, Tufts University School of Dental Medicine, Boston, Massachusetts.* This study investigated initial acceptance or rejection of dental treatment. Matched for age and sex, sixty-six Caucasian first-born children aged three to five and a half years, with no previous dental experience, were divided into groups A and B. Group A's parents were mailed a booklet to be read to the child, along with additional instructions for preparation. A short initial visit, without treatment, introduced the patient to the denist, hygienist, and office. Group B did not receive an introductory visit, nor did the parents receive preliminary information. These children received a dental examination, prophylaxis, and bite-wing X-rays at the first appointment. Group A received this treatment at the second visit. The dentist and hygienist independently rated behavior on a scale. Other factors evaluated were the parents' socioeconomic status and educational level, in addition to the child's apprehension toward a strange environment and previous medical experience. At ages three to four and a half, the initial preparation (group A) significantly increased co-operation. At five to five and a half years, equal co-operation was obtained from both groups. The introductory visit and and additional instructions were found superfluous for group A at this age. Group B children, reported by the parents to be apprehensive toward medical experience, showed the highest degree of non-co-operation at the initial visit.

93. AN EVALUATION OF THE EFFECTS OF SEPARATION OF THE MOTHER AND PRESCHOOL CHILD IN THE DENTAL OFFICE.—*Spencer N. Frankl, Tufts University School of Dental Medicine, Boston, Massachusetts.* One hundred and twelve children aged three and a half to five and a half, without previous dental experience were examined and treated, to study the dynamics of child reaction to separation and non-separation from the mother in the dental office. The purpose of this investigation was to evaluate psychological weaning of the preschool child from the mother as related to children's anxieties associated with dental care. The children were divided into two groups. Group 1 was separated from the mother, and group 2 retained the mother-child pair intact, with the mother instructed to be a passive observer. Children were paired and matched as to age, sex, race, and socio-economic background. Each patient was treated twice, with each appointment limited to 30 minutes. The first visit consisted of an examination, prophylaxis, and roentgenologic survey, while the second involved the administration of local anesthesia and restorative procedures. The child's reaction to dental manipulation was registered on behavior-rating scales by the clinician and two observers. The results demonstrated that children from forty-two to forty-nine months benefited from the mother's presence during treatment. The age group from fifty to sixty-six months showed no differences in behavior with the mother present or absent. The behavior pattern of females to dental care, in both groups, was superior to the male reaction.

94. CHILD REACTION—A COMPARISON UNDER CONVENTIONAL AND HIGH-SPEED OPERATING METHODS.—*Pallonji M. Bhiladvala, Tufts University School of Dental Medicine, Boston, Massachusetts.* The purpose of this investigation was to compare children's reactions to high-speed and conventional operative procedures in relation to individual patient preference, the need for local anesthesia, and postoperative pain. In order to evaluate the response of children to pain, the galvanic skin-response

amplifier and recorder were used. Forty-eight subjects, seven to ten years of age, were evaluated. Incipient carious lesions on the occlusal surface were selected, in order to standardize the preparation and the reaction of the patient to the procedure. At one visit, two cavities were prepared on both sides, either on the maxillary or mandibular first permanent molars or the second deciduous molars, using the high-speed and conventional handpiece. In no instances were the deciduous and permanent dentitions treated concomitantly. Local anesthesia was used when necessary, as demonstrated by the reaction of the patient and determined by clinical judgment. Each subject was recalled after 3 days and questioned about postoperative pain. The evaluation of the data showed that high speed was preferred by children seven to ten years of age, less anesthesia was required, and postoperative pain was similar with both high-speed and conventional techniques. Deciduous teeth were less sensitive than permanent teeth under these procedures.

95. A Quantitative Study of Bone Regeneration in the Rat Calvarium after Decalcified Bone Matrix Implantation.—*Franklin R. Stickel, School of Dentistry, University of Minnesota, Minneapolis.* Homogenous decalcified organic matrix was implanted in trephine defects of the parietal bone of twenty-eight, 60-day-old littermate albino rats. The same-sized unimplanted cranial defect served as the control site. Defect repair was then evaluated at biweekly intervals for 8 weeks on a gross, histologic, and quantitative chemical basis. On gross examination, 20 of the 28 defects which received organic matrix implants were estimated to be completely repaired, and only 1 defect displayed less than 50 per cent repair. In contrast, complete repair of the 16 unimplanted defects occurred in only 3 instances, with 11 animals achieving less than 30 per cent repair. Repair of the implanted defects was more successful when the donor and recipient animals were of the same sex. The organic matrix was accepted by the host without evidence of a foreign-body reaction. The grafts were gradually resorbed, and new bone was deposited for the repair of the defect. In some sections, fibrous tissue was seen joining graft to bed at the periphery, which was eventually transformed to fibro-osseous and finally osseous tissue. A fibrous membrane bridged the lumen of the defect in the control specimens in which repair was incomplete. The results of the quantitative analyses for each specimen paralleled the gross observations. Also, the average calcium content and average percentages of calcium/dry weight for the experimental specimens at the final sacrifice interval were almost identical with those values for normal bone.

96. The Use of Diffusion Chambers in Bone Resorption Studies.—*Jack G. Dale, Forsyth Dental Infirmary, Boston.* A method of autogenous bone transplantation, utilizing Millipore filter diffusion chambers, has been developed to determine whether it is necessary for cells to contact the bone surface for resorption to occur. A large portion of the right scapula was removed from young rats of the Holtzman albino strain, devitalized by a freezing and thawing procedure to kill all associated cells, and divided into three equal parts: (1) one to be histologically prepared for use as a reference; (2) one to be placed "free" in the subcutaneous tissue of the left side of the animal's back for use as a control; and (3) one to be sealed within a diffusion chamber to be placed in a similar area on the right side. The latter transplant represented a piece of "cell-free" bone within a capsule, with pores, which allowed tissue fluid to enter, but not cells. A recovery period of 2 weeks separated the first operation from the second. Thirty-two animals were divided into eight equal groups, and killed at 1, 2, 3, 4, 8, 12, 16, and 20 weeks. Both the reference material and the transplants were subjected to a number of histochemical procedures. Multinucleated cells began to appear around the control transplant after 1 week, and resorption was well advanced after 4 weeks. After 1 month the experimental transplant, within the diffusion chamber, had a fragmented, "motheaten" appearance, suggesting that resorption was taking place.

97. HYDROCORTISONE AND HEALING AND REPAIR.—*Martin Goldberg and Ralph Callender, College of Dentistry, Howard University, Washington, D.C.* This study was designed to determine the effects of corticosteroids, parenterally and locally administered, upon repair processes and reactions to foreign-body implantations. After anesthesia of dba Rockland mice, a mid-line abdominal incision was made, and the skin and superficial fascia reflected. The area was washed with warm sterile saline. Mice in groups of 10 received injections of cortisone 3 days prior to the operations. This was administered as hydrocortisone in amounts of 0.01 cc/gm body weight; where cortisone was applied locally, a paste of cortisone in alcohol was rubbed daily over the wound. One group of controls received injections of 3/20 cc. of N saline, and the other control group received nothing. Injections and topical administration of cortisone were continued for the duration of the experiment, which was 10 days. A tranquilizer,* in amounts of 3 drops per day was put in the drinking water of the animals following the operations. Upon sacrifice, operative areas were removed for histologic study. All responses were inflammatory and ranged from severe with abscess and fistulas to mild reactions. Gross findings, as observed, correlated with histologic findings. Grossly and histologically, it was found that reactions were more severe to oxidized cellulose and least severe where no implants or injections were made. Where no implants were made and cortisone was administered, reactions were more severe to topical applications.

98. A HISTOLOGIC STUDY OF WOUND HEALING FOLLOWING MUCOGINGIVAL SURGERY.—*Theodore L. West and Arthur Bloom, Department of Stomatology, School of Medicine, Boston University.* Two groups of young pure-bred littermate dogs were used to follow the early postsurgical wound healing of exposed alveolar bone. The molars were notched at the gingival margin, and the width of the attached gingiva was measured. The soft tissue was stripped from the buccal bone in a molar area on four separate occasions for each dog. The periosteum was removed on some of the dogs. The exposed bone was measured and covered with Telfa,† surgical pack, and acrylic. The dressing remained in place until sacrifice. The jaws were fixed in 10 per cent formalin, decalcified in 5 per cent nitric acid, and imbedded in paraffin. Healing was studied at 1, 2, 3, 4, 6, 10, 14, and 21 days by means of linear measurements, histology, and photography. Sections were stained with hematoxylin and eosin, Masson's trichrome, and Foote's silver stain. Two maxillary and 2 mandibular specimens were available from different animals for each time period. The granulation tissue was found to migrate apically from the superior wound margin. Where the periosteum was removed, the exposed buccal bone was rapidly and completely resorbed to the wound base. Cemental resorption was occasionally found. Rapid osteogenesis was seen in neighboring areas. The granulation tissue filled the wound and attached to the tooth. At 21 days, epithelialization began at the wound margins. If the periosteum was left intact, the buccal bone was only partially resorbed, and healing was more rapid.

99. A DEFINITIVE MICRORESPIROMETER TECHNIQUE FOR STUDY OF HUMAN GINGIVAL TISSUE.—*A. R. Volpe, J. H. Manhold, Jr., and T. E. Bolden, College of Medicine and Dentistry, Seton Hall College, Jersey City, New Jersey.* Manhold, Bolden, and Katz (*J. D. Res.*, **39**:746, 1960 [Abstr.]) presented preliminary findings of the applicability of a microrespirometer technique to the study of human gingival tissue respiration. Their results, 1.39 ± 0.46 (dry weight Q_{O_2}) or 0.28 (wet), employing an unmodified Stern-Kirk microrespirometer, differed rather widely from those obtained by Senter, Eiler, and Lee (1959) who employed a modified microrespirometer in conjunction with the Kjeldahl (N_2) procedure (1.0 reported wet; 5.0 estimated dry). Because the microrespirometer appears to be ideally suited for study of gingival tissue metabolism (re-

* Atarax®, J. B. Roerig & Company, New York.

† Bauer & Black, Division of Kendall Co., Chicago.

quiring 10 mg. or less wet-weight tissue versus 40–50 mg. for Warburg), further investigation appeared worthwhile. Resultantly, both unmodified (Manhold, Bolden, and Katz), and modified (Senter, Eiler, and Lee) microrespirometer techniques were used in this study. (Kjeldahl study was eliminated because results are readily obtainable directly from the microrespirometers.) Results show (1) The preliminary 1.39 ± 0.46 (dry) Q_{O_2} reported by Manhold *et al.* appeared to hold relatively constant, i.e., 1.44 ± 0.44 for 55 samples. These results also appear in accord with those obtained by use of the well-standardized Warburg manometric technique in gingival tissue study, i.e., 1.60 ± 0.37 (Glickman, Turesky, and Hill); 1.30 ± 0.07 (Glickman, Turesky, and Manhold); 2.4 (estimated dry; 0.48 reported wet) (Schrader and Schrader). (2) There was no statistically significant difference between results obtained by use of the modified and unmodified microrespirometers (critical ratio = 0.81). (3) A standardized procedure for use of microrespirometers in studying metabolism of gingival tissue samples is feasible.

100. Histopathologic and Histochemical Observations in *DL*-Ethionine-Treated Rats.—*John H. Biggs, Medical College of Virginia, Richmond, Virginia.* Forty-one young albino rats were divided into three groups. Two groups received daily intraperitoneal injections of 25 or 50 mg. of *dl*-ethionine in aqueous suspension. Control Group III received daily injections of isotonic saline. Members from each group were sacrificed, beginning on the tenth day of treatment and every fifth day thereafter until 1 month of treatment was completed. Sections of liver, pancreas, and salivary glands were prepared, utilizing both freeze substitution techniques and other standardized laboratory procedures. The liver demonstrated increasing intracellular accumulations of both neutral fats and phospholipids. This change was correlated with a depletion of glycogen storage, as revealed by periodic acid–Schiff staining methods and substantiated by comparison with sections predigested in diastase prior to staining. The pancreas demonstrated disorganization of acinar tissues and replacement by fat. In later stages of degeneration there was an infiltration of cells resembling macrophages that contained a material stainable by PAS methods but not hydrolyzed following treatment with diastase. A similar infiltration was observed in the interlobular connective tissues of the liver. Sections of degenerating pancreas stained with toluidine blue revealed a heavy infiltration of mast cells in some instances. Alkaline phosphatase activity was confined primarily to blood vessels and perivascular areas. A study of salivary glands revealed no particular changes over the normal appearance.

101. Electron Microscopy of Periodontal Disease.—*Cliff Taylor, College of Dentistry, Baylor University, Dallas, Texas.* Patients who exhibited chronic periodontitis with moderate to severe bone involvement were selected. At the time of periodontal surgery, tissues were secured from various sites for examination under the electron microscope. Each tissue was immediately fixed in Palade's solution, then taken through the standard imbedding process. Sections were cut at $1/40\ \mu$ on the ultra-microtome.* Histopathologic study of periodontal disease has been primarily performed under the light microscope. Special attention in this study has been given to types of cells present, pathology of the vascular system, and pathology of the supporting structures. At the sites where there are aggregations of inflammatory cells in the lamina propria, the covering epithelium exhibits intercellular and extra-cellular edema, with stretching of the tonofibrils. The collagen fibers of the gingival group are progressively replaced by dense inflammatory infiltration, which produces the loss of stippling in the attached gingival. In the areas of diffuse collagen destruction there are aggregations of multinucleated cells and cells resembling plasma cells. There is an apparent disruption in the continuity of the endothelial cells and partial or complete occlusion of the vessels in the area of inflammatory cell infiltration.

* Porter Blum.

IX. PHARMACOLOGY. MARCH 24, AFTERNOON, SECTION A

102. CLINICAL AND HEMATOLOGIC OBSERVATIONS ON CHILDREN TAKING AN ORAL IRON PREPARATION.—*H. William Watts, Ralph E. McDonald, and Joseph C. Muhler, Indiana University School of Dentistry, Indianapolis.* Previous work concerning the effects of orally-taken iron preparations on enamel pigmentation are inconclusive. This study concerned itself with the clinical and hematologic evaluation of a new iron preparation consisting of an iron carbohydrate complex. Two groups of children with different caries susceptibility were used. The treatment group consisted of 25 children, the control group of 19 children. There is evidence indicating that carious teeth or etched areas are more readily pigmented than sound teeth. This resulted in the use of four groups of children, two of which were in the appropriate control group. The comparative groups had the same caries expectancy. Hemoglobin and red blood-cell counts were made on all children once prior to initiation of the iron preparation and at monthly intervals throughout a 90-day study period. Kodachrome photographs of the teeth were taken before the study began, once during the study, and at the termination. The results of the iron study showed totally an increased red-blood-cell count in 20 children; increased hemoglobin in 10; no change in hemoglobin in 4. There were no side effects noted and no pigmentation of the teeth.

103. EFFECTS OF ANALGESICS AND PLACEBO ON POSTSURGICAL PERIODONTAL PATIENTS.—*Henry S. Brenman and D. Walter Cohen, Department of Periodontology, Graduate School of Medicine, University of Pennsylvania, Philadelphia.* Following gingivectomy and first change of dressing, 830 doses of six capsules of medication were dispensed. Six capsule doses of either placebo, Zactirin® compound, or Phenaphen-Codeine® compound were dispensed on a double-blind randomly selected basis. Most patients received all these medications at either a surgical or a change-of-dressing visit. Each medication group was packed in identical opaque green capsules, and a master sheet of randomization was utilized to label compounds and placebo bottles containing six capsules with letters A through K. Dose labeling was handled by a disinterested third party. Dose code was not known to investigators or patients. Each patient was assigned a code sheet with four code letters. Following a surgical and/or a change-of-dressing visit, the medication was dispensed according to a quartet of letters obtained from the master code sheet. Each patient, upon his subsequent visit, was questioned by one of the investigators according to a printed routine. All answers following four visits (2 surgical, 2 change of dressing) were contained on one sheet.

Of the 830 patient-visit doses dispensed, 390 were not needed, as pain was not present. Of the 440 patient visits, 62 per cent obtained complete relief with the Phenaphen-Codeine® preparation, 56 per cent with the Zactirin® preparation, and 44 per cent with placebo. Side effects of Phenaphen-Codeine were 26 per cent, Zactirin 15 per cent, placebo 8 per cent. A statistical analysis of the records was completed.

104. DISTRIBUTION OF TETRACYCLINE HCl IN THE GRANULOMA POUCH.—*Daniel M. Laskin, Frank L. Lock, and Milton B. Engel, College of Dentistry, University of Illinois, Chicago.* To disclose whether tetracycline can penetrate or not into an abscess, its distribution in the granuloma pouch wall was studied by fluorescence microscopy, and its concentration in the exudate determined photofluorometrically. Seven days after formation of the air-turpentine pouch, the rats were given either 25 or 100 mg/kg body weight of the antibiotic intravenously. They were then sacrificed at intervals from 5 minutes to 96 hours. Tissue specimens were frozen-dried for microscopic examination, and the tetracycline in the purulent exudate was extracted by Kohn's method and measured in a photofluorometer. Exudate was also tested for bacteriostatic activity against *Staphlococcus aureus* 209P. Tetracycline was visible in the pouch wall 5 minutes after injection and persisted for 48 hours. It was first noted in blood vessels and leukocytes, then in the ground substance and fibroblasts. Measurable amounts of tetracycline appeared in the exudate in 5 minutes and reached

peak concentrations in 12 hours with low dose and in 6 hours with the higher dosage. Bacteriostatic concentrations were attained only at 12 hours, with the static concentrations attained only at 12 hours with the smaller dose, but present from 5 minutes through 24 hours with the larger amount. These results indicate that tetracycline can readily enter inflamed areas, that it remains for relatively long periods, and that it rapidly reaches bacteriostatic levels when large amounts are given. The distribution pattern of the antibiotic and its concentration in specific areas of the granuloma pouch wall appear to be related in part to binding with the serum and tissue proteins.

105. Effectiveness of L-67 and Lidocaine as Block Anesthetics.—*P. E. Tullar and E. W. Roberts, University of Texas, Dental Branch, Houston.* A local anesthetic resembling lidocaine (L-67 Astra) has been used with dilute epinephrine under clinical conditions and found to produce mandibular block anesthesias adequate for many procedures in operative dentistry, extractions, etc. In this double-blind study, anesthesias with L-67 in 2 and 3 per cent solutions plus epinephrine in 1:200,000 and 1:300,000 dilutions were compared with anesthesias obtained with 2 per cent lidocaine with 1:200,000 epinephrine, and with 2 per cent lidocaine with no vasoconstrictor. Operative procedures began within 5 minutes of injection except in those instances where anesthesias were inadequate or failed to last for the duration of the operative work. These were recorded as failures, and the dental work was completed under another anesthetic preparation. Effective anesthesias were produced in 95 per cent (40) of the patients receiving L-67–epinephrine solutions, compared with the effective anesthesias obtained with 91 per cent (31) of those receiving 2 per cent lidocaine with epinephrine 1:200,000; and with the 59 per cent (13) of those receiving 2 per cent lidocaine plain. Thus L-67 with epinephrine offers the same advantages in onset, duration, effectiveness, and freedom from toxic or allergic manifestations as did lidocaine with epinephrine in corresponding concentrations. The high incidence of failures in the group receiving no vasoconstrictor benefits emphasizes the importance of the added vasoconstrictor: and the high percentage of successful anesthesias following L-67 with epinephrine 1:200,000 and 1:300,000 indicates that adequate anesthesias for many procedures can be achieved with from one-half to one-sixth the 1:50,000 epinephrine concentrations sometimes employed.

106. The Effect of Vasoconstrictors on the Cytotoxicity of Dental Local Anesthetics.—*James M. Jablon and Doran D. Zinner, National Children's Cardiac Hospital, Miami, Florida.* In a previous study, tissue-culture techniques were adapted to investigate the comparative cytotoxic effect of various dental local anesthetics. Monolayer cultures of HeLa cells and monkey kidney cells were used, and the cytotoxic effects were estimated on the basis of viability staining, using trypan blue to differentiate between living and dead cells. The results indicated that there was a marked difference between the cytotoxicity of the various anesthetics on the basis of the technique employed. These results, however, were only semi-quantitative, and improved techniques were sought to improve the quantitative character of the results. During the course of the investigation, 3 preparations of 1 anesthetic were studied; these preparations varied only in their concentration of epinephrine. It was noted that the preparations with epinephrine showed less toxicity than did the preparation without epinephrine. The present study was designed to investigate further the effects of epineprine and other vasoconstrictors on the cytoxicity of various dental local anesthetics, using improved techniques to give better quantitative results. Monolayer cultures were trypsinized, and suspensions of cells were prepared. Dental local anesthetics, with and without vasoconstrictors, were tested for their comparative cytotoxic effects on the cell suspensions. Trypan blue was then added to differentiate between living and dead cells, and cell counts were made on a hemocytometer. The results indicate that the addition of vasoconstrictor reduces the toxicity of most of the

anesthetics studied. The degree of toxicity reduction, however, is not constant but varies with the different anesthetics used in the study.

107. Destruction of Local Anesthetics in Blood from Humans. II.—*J. Roy Doty, John J. Hefferren, and Henry M. Koehler, Division of Chemistry, Council on Dental Therapeutics, American Dental Association, Chicago, Illinois.* As part of a continuing study on the enzymatic degradation of local anesthetics, in vitro studies were made of the rate of enzymatic degradation of ester- and amide-type agents in defibrinated blood and serum. Milligram quantities of local anesthetic agents were incubated with known volumes of blood or serum for a predetermined period of time. The incubated mixture was made alkaline with a solution of trisodium phosphate, and the liberated base was extracted with dichloroethane. The ester-type local anesthetics were re-extracted into 0.1 N hydrochloric acid and determined spectrophotometrically in acid or buffered solutions. The amide local anesthetics were determined colorimetrically in an organic solvent mixture by reaction with cis-aconitic anhydride, a method which is specific for tertiary amines. The ability to recover the local anesthetics from water, defibrinated blood, and serum has been demonstrated, and the extent of simultaneous hydrolysis of local anesthetics has been determined. The rates of enzymatic degradation of metabutethamine, butethamine, tetracaine, procaine, lidocaine, and mepivacaine hydrochlorides in definbrinated blood and serum will be reported and compared with earlier studies using whole blood and serum.

108. Meperidine and Promethazine Hydrochloride for Handicapped Patients.—*Manuel M. Album, Medical Arts Building, Jenkintown, Pennsylvania.* Fifty patients with cerebral palsy and mental retardation were given a combination of 25 mg. of meperidine and 25 mg. of promethazine hydrochloride to reduce apprehension prior to dentistry. Saline solution and 50 mg. of meperidine were used as controls. All medication was packaged in separate coded vials unknown to the investigators. A different vial was used each visit. Comments were analyzed when the code was broken upon completion of the study. Intramuscular administration was used on all patients. Degree of retardation and emotional instability influenced the quantity of medication used. Hyperkinetic activity required larger doses. Epilepsy affected actual drugs used. Marked tranquilizing effects were observed with combination of meperidine and promethazine hydrochloride. Five additional retarded patients received the combination of meperidine and promethazine hydrochloride intravenously. This was administered by the hospital anesthesiologist on an out-patient basis. Past dental experiences influenced the medication. The introduction of a barbiturate prior to the intravenous medication was necessary to produce complete relaxation of the patient. These patients throw off their medication rapidly following induction. Sixteen normal children received known doses of the drug. Half the amount of this medication produced the same results as those obtained by larger doses of meperidine.

109. Effect of Diphenylhydantoin Sodium and Salicylate on the Periodontium of Hamsters.—*P. H. Staple, University of Alabama Medical Center, Birmingham.* Because it has been shown that systemic effects of diphenylhydantoin sodium* may be modified by salicylates, these drugs were administered in combination to see whether an oral reaction could be elicited, despite a recent report that dilantin alone was ineffective. In all, forty male animals, about 120 gm. weight, after 1–2 weeks on a powder diet alone were randomly allotted to four groups and then treated daily for 6 weeks as follows: Group I, dilantin sodium, 25 mg/kg i.p.(D); II, sodium salicylate, 60 mg/kg s.cut. (S); III, D plus S; IV, NaCl,Na • i.p. ≡ D,Na • s.cut. ≡ S. Hyperplasia of buccal gingivae in maxillary molar regions was found in some of I and III but not in II or IV, which showed only lesions typical of animals on powder diet alone. Present data do not show whether salicylate potentiated the effect of dilantin on the

* Dilantin sodium.

periodontium. Between groups, mean maxillary alveolar bone resorption scores did not differ significantly; but comparison of variances indicated that I and II could not belong to the same population as III and IV ($F18, 16 = 4.56$), a finding at present inexplicable. The specificity of the gingival response is being further investigated by drug-withdrawal experiments in which gingival thickness measurements on casts will be made, together with determinations of the tensile strength of healing skin wounds.

110. Cell-stimulatory Effects of Diphenylhydantoin Sodium in Tissue Culture.—*William G. Shafer, Indiana University, Schools of Dentistry and Medicine, Departments of Oral Pathology and Pathology, Indianapolis.* Previously reported studies from these laboratories have shown an increase in tensile strength of healing wounds in rats administered diphenylhydantoin sodium* and stimulation of proliferation of a human gingival fibroblast-like cell in tissue culture by dilantin. Several analogues of dilantin have also been tested, as well as a number of structurally related compounds, including uric acid, urea, allantoin, hydantoin, alloxan, and barbital sodium for similar stimulatory properties. Of all these materials, only two dilantin analogues (1-allyl-5-phenyl hydantoinate and 5-methyl-5-phenyl hydanoinate) were effective. Because of this unusual effect of dilantin on a human gingival fibroblast-like cell, studies have been carried out on other cell lines maintained in this laboratory, including (1) normal adult mouse heart, (2) normal adult mouse kidney, (3) mouse embryo fibroblast, (4) rat fibrosarcoma, (5) rat rhabdomyosarcoma, and (6) mouse salivary gland tumor (BW1081). Of all cell lines tested, only the BW0181 tumor showed as dramatic a stimulation of cell proliferation as the human gingival fibroblast-like cell by dilantin. This was evidenced by a twofold increase in the number of cells in dilantin-treated culture tubes as contrasted with control tubes at the 4-day incubation period. Evidence suggests a rather specific activity-response effect.

111. Parathyroid Hormone and Serum Mucoprotein Levels.—*Ernest Hausmann, School of Dentistry and School of Medicine, University of Buffalo.* Several recent studies have exhibited a rise in serum mucoprotein fractions after administration of prepared parathyroid extract (PTE)† to rats. It has been suggested that these elevations were a manifestation of mucopolysaccharide breakdown of bone matrix subsequent to enhanced bone resorption. Were one to assume that demineralization of bone must precede any attack on the matrix, a view shared by the Neumans (*Chemical Dynamics of Bone Mineral*) in their summation of available evidence, it may be shown that the rise in serum mucoprotein observed after PTE could not have originated from bone matrix. One can calculate, within reasonable limits, that the increase in serum which would have to accompany that observed mucoprotein-carbohydrate would be fantastically high. On the basis of values in the literature for the size of the extracellular fluid space, contribution of mucopolysaccharide and calcium to the total weight of bone, and excretion of calcium after PTE, one can calculate that the rise in serum mucoprotein-carbohydrate observed by Engel (*Arch. Pathol.*, **53**:339, 1952) 24 hours after PTE administration would have to be accompanied by a serum calcium rise close to 3,000 mg. per cent. Similarly, the rise in seromucoid-carbohydrate of 10 mg. per cent, observed by Shetler *et al.* (*Am. J. Physiol.*, **195**:535, 1958) would have to be accompanied by a serum calcium increase of about 1,800 mg. per cent. It is suggested, therefore, that the extraneous material in PTE influences production and/or destruction of serum mucoprotein by tissues other than bone. The problem of the relationship between parathyroid activity and serum mucoprotein levels may well be resolved by studies utilizing highly purified parathyroid hormone.

112. Comparative Cardiac Toxicity of Local Anesthetics.—*I. M. Fraser, N. P. Markussen, J. E. McKinster, and J. Hayden, School of Dentistry, College of Medical Evangelists, Loma Linda, California.* Eleven local anesthetics commonly employed in dental practice have been compared in regard to their cardiac toxicity in dogs by

* Dilantin sodium.

† Eli Lilly.

studying their effect on the electrocardiogram when injected intravenously in minimal doses. Using the standard limb leads, it was found that all the agents tested decreased the amplitude of the *R* wave and usually increased the amplitude of the *T* wave. The depression of the *R* wave was quantitatively related to the dose of the local anesthetic over the lower range of dosages. The dosage required to produce a 20 per cent depression of the *R* wave has been determined for each agent, thus making possible a comparison of the relative toxicity of each compound to procaine. In general, the results were in agreement with data obtained by others from intravenous acute toxicity determinations in mice and other animals.

113. Effect of Adrenocorticotropic Hormone on the Osseous System of Rats.—*Hermann Becks and J. O. Riebe, Division of Oral Biology, School of Dentistry, and the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.* A study was made of histologic changes in endochondral bone formation in tibias and the healing of postextraction alveolar sockets in rats treated for varying time intervals with long-acting ACTH.* Only changes in the tibias will be discussed. Twenty-six female rats (Long-Evans strain), age 55 days at onset, were segregated into 6 groups to be sacrificed at 65, 68, 71, 74, 78, and 82 days of age. The experimental animals of each group received daily subcutaneous injections of 16.0 U.S.P. units Cort-Zn from age 55 days until autopsy. A mandibular third molar was extracted from all control and experimental rats at age 60 days. Female rats injected with ACTH displayed marked weight loss, and retardation in growth. A reduction in chondrogenesis in the proximal epiphyseal disk of the tibia was reflected by distinct narrowing in the width of the cartilagenous disk due to decreased size and number of cartilage cells and intercellular matrix. Metaphyseal trabecular architecture in the experimental tibias was one of irregular, increased density in those rats injected for 9 days, but progressed to an orderly, reduced density in animals injected for longer periods of time. The absence of osteoblasts and osteoclasts on the trabecular, endosteal, and periosteal surfaces suggests a reduction in osteogenic processes and no increment in osteolytic sequences. Possible fatty infiltration of the marrow was noted in later specimens.

114. Fetal Uptake of Orally Administered Radio-Calcium in Rats.—*James W. Bawden, Radiation Research Laboratory, State University of Iowa, Iowa City.* The study was designed to investigate the transfer of orally administered calcium-45 from mother to fetus by use of accurate quantitative assay procedures. A series of female Holtzman rats were placed with males and checked each morning for mating activity. The sperm-positive females were given 0.5 ml. of a standard calcium-45 solution by stomach tube each day of gestation. This solution contained approximately 1 mc/ml. The newborn animals were weighed, ashed, and the ash was dissolved in hydrochloric acid solution. Aliquots from the standard solutions and from the fetal ash solutions were assayed, using a windowless gas-flow counting system. The percentage of the total administered dose recovered in the fetal tissues was 4.04, with a standard deviation of 0.92. Changes in transfer when the mother is subjected to conditions of calcium stress during pregnancy will be discussed.

X. HISTOCHEMISTRY. MARCH 24, AFTERNOON, SECTION B

115. Polysaccharide Histochemistry of the Tooth Germ.—*Giuliano Quintarelli, University of Alabama Medical Center and University of Rome Medical School, Italy.* The distribution of some mucopolysaccharides and glycoproteins in the tooth germs and nasal cartilage of newborn rats was studied. After fixation, sections were stained with PAS, alcian blue–PAS, colloidal iron, colloidal iron–PAS, colloidal iron–Feulgen, azure A, and safranin O. Prior to staining, sections were incubated in hyaluronidase, pepsin, and papain. A 20-minute incubation in pepsin partially decreased

* Cortrophin-Zinc, Organon, Inc., Orange, New Jersey.

metachromasia, while papain digested all the chromotrope material from the dental papillae and the dentin. Different were the results when special stainings for mucopolysaccharides were used. Although incubations in hyaluronidase decreased the staining intensity, some alcianophilic material was not digested. These findings suggested that either the chemical linkage between alcian blue and the acid radicals is different than that between azure A and the reactive sites of the polysaccharides or that another substrate is present and stained only with alcian blue or colloidal iron. In other investigations it was found that sialic acid is readily stained with alcian blue but not with azure A. In view of the recent findings of Amici *et al.* on the presence and high content of this sialomucin in the dental pulp and in the dentin, the results obtained may indicate the following: in the dental papilla and in the dentin of newborn rats, besides the presence of acid mucopolysaccharides, there may also exist a sialic acid–containing glycoprotein.

116. Demonstration of Dehydrogenases in Tooth Buds of Newborn Rats.—*Albert I. Farbman, Edward J. Reith, and E. D. Goldsmith, College of Dentistry, College of Medicine, and Graduate School of Arts and Science, New York University, New York.* Dehydrogenase enzyme systems were studied in the tooth buds of newborn rats. Incisor tooth buds were removed from the jaws and placed immediately in incubating media for 30 minutes at 37° C. Test media contained sodium succinate or sodium malate plus DPN as substrate, phosphate buffer at pH 7.2–7.4, neotetrazolium phosphate, potassium cyanide, and phenazine methosulfate. Two classes of controls were used. One included prior fixation in 10 per cent formaldehyde, and the other was incubated without prior fixation in a medium containing no substrate. After incubation, the tissue was immersed in 10 per cent formaldehyde to stop the reaction. Tissues were then infiltrated with gelatin and sectioned in a cryostat. In addition, fresh-frozen sections were made and then incubated in test and control media identical with those mentioned above. Preliminary observations reveal the presence of succinic and malic dehydrogenase in ameloblasts, in addition to odontoblasts and stratum intermedium.

117. Distribution of Phosphorylase in Oral Mucous Membrane.—*Jules Klingsberg and Earl O. Butcher, Department of Anatomy, College of Dentistry, New York University.* Fresh-frozen sections of mandibular and maxillary molar gingivae, palate, tongue, and vestibular mucosa of 60 Long-Evans rats, 3–720 days of age, were stained for phosphorylase and amylo-1,6-transglycosylase by the method of Takeuchi. A longer period of incubation was required to achieve maximum staining in tissues of older animals, although evidence of phosphorylase activity was noted in all the tissues studied. The location of the activity varied in different aged individuals. Within 20 minutes, all but the most external layers of the epithelium of 3–7-day-old animals stained purple, which is indicative of phosphorylase activity by this technique. This reaction was confined to the basal layer and lowermost prickle-cell layer in older animals and required 45–90 minutes of incubation. Amylo-1,6-transglycosylase was always observed in both the basal and the spinous layers of the epithelium of all ages. Amylo-1,6-transglycosylase was most abundant in the marginal gingiva and the palate, which had previously been reported to be glycogen-positive. Phosphorylase activity was observed in these regions but was more abundant in the basal cells of the attached gingiva and palate and the most apical portion of the crevicular gingiva. Glycogen was usually not found in these areas. The usual finding of glycogen in the prickle-cell layer may be due to decreased rate of utilization, since there appears to be decreased enzyme activity in this area. Similarly, the usual lack of glycogen in normal basal cells, where phosphorylase is abundant, suggests rapid utilization.

118. Epithelial DNA and RNA Synthetic Activities of the Gingival Margin.—*Richard C. Greulich, Department of Anatomy, School of Medicine, University of California at Los Angeles.* An investigation of DNA and RNA synthetic activities has been undertaken, with partciular regard to the epithelium of the gingival margin

and attached epithelial cuff. Male and female mice at 90 days of age were given single or multiple intraperitoneal injections of either tritiated thymidine or tritiated cytidine in amounts ranging from 10 to 60 μc. Animals were sacrificed serially thereafter at intervals ranging up to 6 days. Maxillary and mandibular dentition, together with adjacent soft tissues, was decalcified in EDTA at pH 7.4 following Bouin fixation. Paraffin sections wert autoradiographed according to the "dipping" method of Messier and Leblond. Exposures ranged from 7 days to 3 months. Examination of thymidine autoradiograms has revealed that DNA synthetic activity and presumably, therefore, mitotic activity in the region of the gingival margin and attached epithelial cuff is considerably greater than that of adjacent oral mucosal epithelium. Moreover, the synthesis of RNA in these cells, as determined by their cytidine uptake, is also very much more marked in paradental regions than in oral mucosa generally. These findings support the concept that the tissues of the gingival margin and attached epithelial cuff are physiologically modified to perform supportive and protective functions specifically associated with the teeth and should be considered to be functionally separate from the oral epithelium as a whole.

119. Histochemical Aspects of Gingival Disturbances.—*Peter D. Ferrigno and B. R. Bhussry, School of Dentistry, Georgetown University, Washington, D.C.* This investigation was designed to characterize some of the clinical changes in various forms of gingival disturbances by means of histologic and histochemical techniques. The gingival tissue for this study was obtained from patients in the Department of Periodontia. Patient history, clinical examination, and full-mouth X-rays were obtained. Color photographs were made to maintain a record of the clinical appearance of the gingival tissue prior to biopsy. The gingival biopsy was taken, and the tissue was fixed in neutral formalin. It was imbedded in paraffin by routine procedures. Serial sections were prepared and stained with hematoxylin and eosin stain, Masson trichrome stain for connective tissue, and Verhoeff's stain for elastic tissue, the PAS for polysaccharides, toluidine blue for metachromasia, and Muller's technique of Hale's iron absorption for demonstration of acid and neutral mucopolysaccharides. The observations so far suggest a marked increase in the fibrous elements of chronically inflamed gingival tissue. No differences could, however, be noted in the amount of elastic tissue in these cases. Alterations in the amount of mucopolysaccharides were demonstrated by metachromatic reaction and also by Muller's technique.

120. A Histochemical Study of the Relationship of Oxytalan to Elastic Fibers.—*Harold M. Fullmer, National Institute of Dental Research, National Institutes of Health, U.S. Public Health Service, Department of Health, Education, and Welfare, Bethesda, Maryland.* Oxytalan and elastic fibers in the periodontium of the mouse, rat, guinea pig, rabbit, deer, steer, hog, dog, sheep, monkey, and man were studied. All specimens were fixed in neutral 10 per cent formalin, decalcified in 5 per cent formic acid, and imbedded in paraffin. Elastic fibers were identified by the orcein and orcinol-new fuchsin stains. Oxytalan fibers were demonstrated by the peracetic acid–aldehyde fuchsin–Halmi and the peracetic acid–orcein-Halmi stains. Oxytalan fiber demonstration was further verified by the abolishment of the staining reaction by either a testicular hyaluronidase or a β glucuronidase digestion; in addition, these digestions were prevented by known specific inhibitors. All, or nearly all, of the non-collagenous fibers of periodontal membranes of the mouse, rat, guinea pig, monkey, and man were of the oxytalan type. On the other hand, many elastic fibers were found in the gingivae and coronal thirds of the periodontal membranes of the rabbit, deer, steer, dog, sheep, and the hog; however, the non-collagenous fibers about the middle and apical thirds of these teeth were either all or nearly all oxytalan. This study suggests a phylogenetic relationship of elastic and oxytalan fibers.

121. Histologic and Histochemical Studies of Salivary Gland Growth.—*Chester S. Handelman and Herbert Wells, Biological Research Laboratories, Harvard*

School of Dental Medicine, Cambridge. A rapid and marked increase in the rate of salivary gland growth in rats follows amputation of the lower incisor teeth. Histologic and histometric studies of the enlarged glands show that the increased gland growth is due to hypertrophy of the acinar cells. There is no change in the number of acinar cells. While the size and number of tubules and excretory ducts are unaltered in enlarged glands, there is a striking depletion of the granules usually found in the tubules. Treatment with dibenamine, an adrenergic blocking agent, not only blocks the response to incisor amputation but results in a marked atrophy of the salivary glands. Histologic and histometric examination of the atrophic glands reveals the acinar cells to be markedly reduced in size, while the size and number of tubules and excretory ducts are unchanged. Therefore, the atrophy of the salivary glands following treatment with dibenamine appears to be due entirely to decrease in size, without alteration in number, of the acinar cells. Histochemical studies show a marked increase in the RNA content of hypertrophied acinar cells and a decrease in RNA content of acinar cells made atrophic by dibenamine. Periodic acid–Schiff and alcian blue stains show that the mucoprotein and acid mucopolysaccharide content of acinar cells is directly proportional to cell size. It is suggested that the changes in salivary gland growth which follow amputation of the teeth and treatment with dibenamine may provide a valuable tool for elucidating the mechanism of salivary gland growth under normal conditions.

122. Histochemistry of Experimentally Induced Fibrosarcoma in Rat Submaxillary Gland.—*Howard H. Chauncey and Gerald Shklar, Tufts University School of Dental Medicine, Boston, Massachusetts.* 3,4-Benzpyrene (0.3 mg.) was injected into the left submaxillary gland of 48 (24 males, 24 females) 4-month-old albino rats, Wistar strain. Twelve animals (6 males, 6 females) served as controls and received injections of 0.1 ml. of vehicle only (corn oil). The animals received from one to twelve injections. Those receiving multiple applications of the carcinogen were given the material at biweekly intervals. Upon sacrifice, the glands were removed and one half of each gland was fixed in formalin and subsequently stained with hematoxylin and eosin. The remaining portion was quick-frozen and stored at $-10°$ C. until a histochemical evaluation could be initiated. Enzymes studied were acid phosphatase, non-specific esterase, β-*D*-galactosidase, β-glucuronidase, and leucine aminopeptidase. Examination of the tissues revealed the following reactions: normal tissue, chronic inflammation, chronic inflammation and tissue hyperplasia, early fibrosarcoma, and advanced fibrosarcoma. Control animals exhibited no malignancies. Ten of the experimental animals developed fibrosarcomas. Microscopic examination indicated that the material administered was contained within the parenchyma of the submaxillary gland. Variation in the glandular enzyme activity pattern was noted as the tissue underwent changes which eventually resulted in the formation of a malignant neoplasm. There was an increase in the nonspecific esterase and β-*D*-galactosidase activity of the connective tissue fibers. Leucine aminopeptidase activity was found to increase in both the fibers and the fibroblasts.

123. Histochemical Observations of Rat Teeth Following Therapeutic Alterations of Thyroxin.—*Barry G. Miller, Charlotte Memorial Hospital, Charlotte, North Carolina.* Graduated dosages of thyroxin 5–25 μg/d were injected intraperitoneally, using young rats at birth through 16 days of age. Animals were sacrificed in pairs at random, control, and experimental at 1–16 days from 10 litters. The sacrifice tissue was prepared by sectioning the heads with an unused safety razor blade and the mandibles dissected. These tissues were prepared by freeze-dry double-imbedding technique. Mineralized sections accomplished for observation at 5, 7, and 10 μ. Observations for gross changes, e.g., opening of eyes, ears, and tooth eruption, were accelerated, as has been reported by earlier workers. Using these techniques and these tissues, no differences were observed within the ameloblast and odontoblast between the control group and the experimental group in sulfhydryls, disulfides, and polysaccharides.

124. HISTOCHEMICAL DISCLOSURE OF ENZYME ACTIVITIES ON UNDECALCIFIED TOOTH SECTIONS.—*Vincent F. Lisanti, Institute of Stomatological Research, Sias Research Laboratories, Brooks Hospital, Brookline, Massachusetts.* From several hundred teeth, 5–30 coronal sections ($80 \pm 20\ \mu$) were cut through a single lesion. The sections were incubated with specific substrates for each enzyme system for the demonstration of the following enzyme activities. Positive enzymatic reactions: *Acid phosphatase,* purplish-red color in dentinal tubules in carious dentin, reddish-brown color in the plaque on intact enamel. *Alkaline phosphatase,* purple band in lesion, purple dots in cut dentinal tubules, purple color at dentin-enamel junction, and purple band underneath seemingly "unaffected" dentin. *Total esterase,* bluish-green, indigo color in dentinal tubules in the carious lesion area. *Cholinesterase,* purple color in dentinal tubules at the edge of the carious lesion and at DE junction. *Glucosidase,* large amounts of purple color in dentinal tubules in the carious lesion and some purple color in seemingly "unaffected" dentinal tubules under the affected area. *Galactosidase,* purplish color at edge and in the carious lesion. *Beta-D-glucuronidase,* dark purple at the outer edge of the carious lesion and purple color in dentinal tubules in the lesion. *Succinic dehydrogenase,* heavy purple at the outer edge of the lesion and purple in dentinal tubules. The results show that the enzymatic activities are present (1) in plaque (2) in carious dentin (3) at DE junction, and (4) in the so-called "unaffected dentin area" underneath the clinically seen carious dentin.

125. A HISTOCHEMICAL AND AUTORADIOGRAPHIC STUDY OF THE CALCIFEROUS GLANDS OF THE EARTHWORM.—*G. Bevelander and H. Nakahara, College of Dentistry, New York University, New York.* The common earthworm, *L. terrestris,* exhibits 3 pairs of calciferous glands located in segments X–XIII; under appropriate conditions they elaborate spherules consisting of calcium carbonate. Histologic and histochemical studies show that during the active state of the cells a protein precursor of the spherule is formed, which is in part PAS-positive and metachromatic. During the period of elaboration of the precursor, the mitochondria and Golgi apparatus are prominent cytologic features of the cell. Also during the active state a prominent metachromatic granule appears which is surrounded by RNA-positive particles. The spherules are extruded into a sinusoidal space, where mineralization occurs. The state of the mineral is at first amorphous, later crystallization gradually occurs. Injection of Ca^{45} into the animals shows a rapid uptake in the cells of the gland and subsequent incorporation into the spherules. Injection of S_{35} was followed by appearance in the extra-cellular spherules and also the extra-cellular fluid in which mineralization occurs. In the earthworm, specific glandular cells exhibiting increased metabolic activity elaborate a protein precursor and concentrate calcium. These components are combined in an extracellular region consisting of a protein as well as calcium and a sulfated polysaccharide. The formation of the crystalline product occurs in stepwise fashion, giving rise at first to an amorphous state, subsequently to vaterite, calcium, or a mixture of the latter two species.

126. HISTOCHEMICAL CHANGES IN DEVELOPING TEETH OF VITAMIN-DEFICIENT RATS.—*Enrique Kiguel, School of Dentistry, Georgetown University, Washington, D.C.* To study the calcification of teeth in animals fed vitamin D–deficient diets, two groups of 12 female rats each were used. One group received the Steenbock diet (Ca:P 4:1) and the other was fed the Chick Basal diet (Ca:P 2:1). Half of the animals in each of these litters received 2 doses of vitamin D supplements (approximately 100 I.U. of vitamin D per dose). The animals had been depleted of their vitamin D reserves prior to the experiment. Animals receiving a nutritionally adequate diet were used as controls. The female rats were mated with Chick Basal males and pregnancy determined before housing them in maternity cages. The newborn animals from each group were sacrificed at corresponding intervals of 3, 6, and 10 days of age. Undecalcified and decalcified serial sections of the jaws were prepared. They were stained with

hematoxylin and eosin, Masson trichrome, Von Kossa's reaction for calcium, and Mowry's improved technique for acid mucopolysaccharides, and its combination with the PAS reaction for acid and neutral mucopolysaccharides. The observations suggest that in the Steenbock animals the amount of enamel matrix was deficient. A weak Von Kossa reaction in the wide zone of predentin was noted in all animals (with no supplement). The staining for acid mucopolysaccharides in the predentin tended to disappear. The developing enamel and dentin in Steenbock animals receiving supplements of vitamin D showed considerable improvement. The enamel matrix was of better quality, and the zone of predentin was not so wide. The Chick Basal animals which received a supplement of vitamin D appeared to show metabolic disturbances, which were reflected in their developing enamel and dentin.

127. Histochemical Reactions in the Tissues of the Developing Teeth of "IA" Rats.—*Anna Morse, Harvard School of Dental Medicine, Cambridge.* The inheritable characteristic of failure in bone resorption in the "IA" strain of rats causes abnormal relations of the tissue elements of the developing tooth. Unresorbed bony spicules interfere with the orderly progression of amelogenesis and dentinogenesis. The morphologic abnormalities are accompanied by changes in the localization and distribution of RNA, mucopolysaccharide, glycoprotein, glycogen, and aldehyde-fuchsin–positive material. The principal changes consist of a decrease in enamel and dentin deposition wherever the intracellular histochemical reactions are quantitatively reduced, and a lack of glycogen in the basal pulp.

XI. SALIVA AND SALIVARY GLANDS I. MARCH 24, AFTERNOON, SECTION C

128. Some Factors Affecting the Bacterial Inhibition by Parotid Saliva.—*I. L. Shklair and B. L. Lamberts, Dental Research Facility, Great Lakes, Illinois.* Antibacterial factors are known to exist in parotid saliva; however, the intensity of the antibacterial activity varies among individuals. In order to determine the reasons for this variation, 22 caries-active and 22 caries-immune subjects were studied. Paraffin-stimulated parotid saliva was collected for 5 minutes; the patients were given a 15-minute rest period, and 5 ml. more of parotid collected. The relationship of the flow rate to the quantity of lyophilized solids in parotid saliva was determined for each individual. The lyophilized material was reconstituted with water to either one-tenth its original volume or reconstituted to contain 100 mg of solids/ml. The degree of its inhibitory activity against a test organism, *Lactobacillus arabinosus,* ATCC #8014, was measured. The immune group demonstrated a significantly greater parotid flow rate, as well as an increase in the amount of solids secreted per milliliter and per minute. This was reflected in an increased inhibition exerted by the caries-immune parotid saliva when it was lyophilized and reconstituted on the basis of one-tenth its original volume. However, when the lyophilized parotid saliva was reconstituted so that the test samples of both groups contained an equal amount of solids, 100 mg/ml, there was very little variation in the degree of inhibition of the test organism. This may explain some of the variations encountered when testing for the inhibitory properties of parotid saliva.

129. Clinical Aspects of Infrared Studies of Saliva and Dental Plaque.—*R. B. Wolcott, T. S. Meyer, and M. A. Mazzarella, Dental Research Facility, Naval Training Center, Great Lakes, Illinois.* It has been previously reported that the infrared absorption spectrum of the whole saliva of humans and animals exhibits an absorption maximum at 12 μ and 7 μ. This observation was correlated to, and showed a diminishment with, an increase in caries experience. Speculation was made of its diagnostic value for interpretation of the dental caries status. In this laboratory attempts were made to determine the significance of these peaks. Secretions of the individual glands were studied qualitatively for the presence or absence of this absorbance maximum at 12 μ. It was found that a maximum existed in the parotid secretions of both caries-susceptible and "immune" subjects. This peak was found to disappear,

however, in the infrared spectra of the whole saliva of the highly susceptible subjects. Resting dental plaque and plaque activated by a 30 per cent sucrose rinse were analyzed from various oral areas of highly susceptible and caries-"immune" individuals. No absorbance at 12 μ was noted.

130. SPECTROSCOPIC STUDIES ON HUMAN SALIVA.—*T. S. Meyer, R. B. Wolcott, and I. L. Shklair, Dental Research Facility, Great Lakes, Illinois.* It has been noted that the infrared absorption spectrum of the whole saliva of caries-resistant individuals exhibited an absorbance maximum at 12 μ, which was either absent or diminished in the case of caries-susceptible individuals. Work in this laboratory was concerned with the identification of the component responsible for the absorbance maximum at 12 μ. This component also was found in the parotid gland secretion. After dialysis of parotid gland secretion against water, the component was present in the dialyzable fraction. The component was not soluble in benzene or ethyl ether. The absorbance maximum at 12 μ disappeared upon acidifying the parotid secretion. A shift of the absorbance maximum from 12 to 11.4 μ was noted after heating parotid saliva to 100° C. The infrared spectrum of the inorganic ash of parotid secretion exhibited the same absorbance maximum at 11.4 μ. The whole saliva of caries-resistant individuals and the parotid secretion exhibited an additional absorbance maximum at about 14 μ, which was directly related to the maximum at 12 μ. Infrared spectra of bicarbonates of sodium and potassium also exhibited these individual absorbance maxima, and, when bicarbonates are heated or ashed, carbonates are formed which give an absorbance maximum at 11.4 μ. Bicarbonate solutions show identical behavior in all respects. Therefore, the component of whole saliva and parotid secretion which absorbs at 12 μ in the infrared spectrum was found to be inorganic and was identified as bicarbonate.

131. PHYSICAL VARIATIONS AND pH VALUES OF SALIVA AND PAROTID FLUID.—*M. A. Mazzarella, B. L. Lamberts, and T. S. Meyer, Dental Research Facility, Great Lakes, Illinois.* Previously reported pH values of whole saliva have been contradictory, while values for the pH of unstimulated parotid saliva were uniform. Therefore, parotid fluid from caries-rampant and caries-immune subjects was measured immediately upon excretion. A Beckman model "G" was used with a one-drop glass electrode fitted inside a water jacket to maintain temperature of the specimen. Kleinberg-type antimony electrodes were used for in vivo measurements of the accumulated saliva pool behind the mandibular incisors in an attempt to determine the actual salivary environment of the teeth. The pH was determined periodically: (*a*) in freshly collected droplets of parotid fluid, (*b*) at intervals during parotid storage with varied physical states, and (*c*) upon whole pool saliva after 45 seconds of accumulation. Flow rates for 20-minute collection periods with paraffin stimulation were not significantly different between the two groups. The pH of parotid fluid was found at higher levels for greater rates of flow. Titrations of parotid corroborated previously reported work, which showed pH positively correlated with the amount of bicarbonate. The saliva pool was significantly more alkaline in caries-immune patients. Parotid fluid pH rose with storage. This phenomenon was related to Henry's law for gases dissolved in solutions and explains the reported variance in pH. The pH changes observed in parotid after collection were probably due to the escape of carbon dioxide, which upset the carbonic acid–bicarbonate equilibrium. Infrared spectrum studies showed that this pH rise was related to inorganic components of parotid fluid which were part of a carbonic acid–bicarbonate buffering system.

132. STUDIES OF pH CHANGES AND INFRARED ABSORBANCE OF PAROTID SECRETIONS.—*B. L. Lamberts, T. S. Meyer, and M. A. Mazzarella, Dental Research Facility, Great Lakes, Illinois.* Previous studies at this laboratory identified the component of saliva absorbing at 12 μ as bicarbonate. The pH increase of parotid secretion, upon exposure to air, has been attributed to the change in relative concentrations of carbonic acid and bicarbonate, resulting from the escape of carbon dioxide. Contributions of the

inorganic components of saliva—carbonic acid, bicarbonate, and phosphate—to the pH changes, and the infrared absorbance observed in paraffin-stimulated human parotid secretion were investigated. Solutions of carbonic acid–bicarbonate and of monohydrogen phosphate–dihydrogen phosphate were prepared at concentrations and pH levels comparable to those of parotid secretion. Experiments were carried out at 25° and 37° C. to compare the pH changes of these mixtures, individually and in combination, with those of parotid secretions of caries-rampant and caries-immune subjects. The pH rise of parotid secretion, exposed to air, could essentially be duplicated by mixtures of the inorganic buffering components of saliva. The carbonic acid–bicarbonate system is primarily responsible for the pH rise and infrared absorbance observed. Inorganic phosphate, however, is present in parotid secretion in sufficient concentration to exert a buffering influence on the rate of pH change, an effect which is particularly evident when the bicarbonate:phosphate ratio is relatively low. At the concentration at which inorganic phosphate (e.g., dipotassium phosphate) occurs in the parotid secretion, the contribution of this component to the absorbance maximum at 12 μ is negligible.

133. The Inhibition of Coliphage T_1 by Oral Secretions.—*H. Wolochow and C. E. Meyers, Naval Biological Laboratory, School of Public Health, University of California, Berkeley.* Current studies at this laboratory required the counting of coliphage T_1 in oral secretions obtained by gargle with saline. It was found that the number of particles of phage recovered in such solutions was markedly lower than expected. A number of factors which might influence this activity of oral secretions was studied. The inhibiting substance was heat-stable, not inactivated by chloroform, passed through a membrane filter, and was not precipitable by centrifugation at 40 kg. for 20 minutes. Calcium chloride prevented, but did not reverse, inhibition of the phage.

134. Thromboplastin Generation by Saliva.—*Hristo Chris Doku and Richard G. Taylor, Tufts University School of Dental Medicine, Boston, Massachusetts.* In a previous study it was shown that saliva has a thromboplastic-like activity. The purpose of this study was to determine directly whether saliva has any specific thromboplastic activity by comparing it with commercial tissue thromboplastin or whether its effect in accelerating blood coagulation is non-specific. One-stage prothrombin time determination and thromboplastin generation tests were performed in twenty subjects. First, commercial tissue thromboplastin and then saliva were substituted for serum, adsorbed plasma, and chloroform-brain extract (CBE), one at a time, in the thromboplastin generation test. The strength of the commercial tissue thromboplastin was varied for 15–35 seconds, as determined by the one-stage prothrombin test. In the case of commercial tissue thromboplastin, it was found that there was no detectable generation at any time or concentration. On the other hand, saliva showed definite thromboplastin generation over a period of time, constantly increasing and then deteriorating. The above findings demonstrate the presence of a specific activity of saliva related to plasma thromboplastin, which is complete and also presents a serum-like activity. Similar activity was not demonstrated in the commercial tissue thromboplastin.

135. Effect of Sialoadenectomy on Growth and Body Composition of Rats.—*Winfrey Wynn, John Haldi, and M. L. Law, Emory University School of Dentistry, Atlanta, Georgia.* It has been reported previously from our laboratories and from the laboratories of others that albino rats that have had their major salivary glands removed do not grow as well as their littermate controls. This has been shown to be true in both ad libitum and pair-feeding experiments. Even with the best of care, the animals spill some of their food. This, of course, invalidates comparison of food intakes. To overcome this variable, 20 littermate pairs of rats were fed ad libitum until they reached 100 gm. One of each pair was then sialoadenectomized and the other subjected to a sham operation. The animals were then pair-fed for 1 week during recovery from

the operative procedure. For the next 60-day period all animals were fed by stomach tube, both members of each pair receiving the same amount of food each day. At the end of the experimental period, the animals were sacrificed, and aliquots of the body mass were analyzed to obtain the body composition. The average weight gained was 121 gm. for the sham-operated and 103 gm. for the sialoadenectomized animals. This 18-gm. difference in weight was found to be made up of 9.1 gm. water, 5.2 gm. fat, 2.5 gm. protein, and 0.5 gm. ash. There was no appreciable difference in the fecal caloric output or in basal oxygen consumption. These results indicate that sialoadenectomized albino rats do not grow as well as their sham-operated littermates.

136. Effects of Total Inanition on the Submaxillary Gland of the Rat.—*Arnold Tamarin and Leo M. Sreebny, Department of Oral Pathology, School of Dentistry, University of Washington, Seattle.* Fifty-four adult male rats were divided into 9 groups of 6 animals each and starved for the following periods of time: zero, 3, 12, 24, 48, 72, 120, 168, and 216 hours. Body and gland weights were obtained, and histologic sections were prepared. The following measurements were made on photomicrographs of the histologic sections: diameters of convoluted tubules, tubular and acinar cell densities, and nuclear diameters of acinar and tubular cells. The effect of starvation on the submaxillary gland of the rat can be summarized with the following series of statements: (1) The glands lost weight in proportion to the loss of body weight. (2) The loss in weight was due mainly to the shrinkage in size of the tubular and acinar cells, and this progressed with increasing duration of starvation. (3) The diameter of the convoluted tubules likewise decreased with the duration of starvation and was related to the decreased size of the tubular cells. (4) Acinar and tubular cells decreased in size at the same rate. (5) Starvation seemed to have no effect on the size and shape of the acinar and tubular nuclei. (6) Acinar cells demonstrated more profound pathologic changes than did tubular cells for equal periods of inanition. (7) The percentage of body weight lost during starvation was represented by the formula log per cent loss + 0.802 log duration — 0.247.

137. Salivary Gamma Globulin in Oral Health and Disease.—*Frederick W. Kraus, Veterans Administration Hospital, Birmingham, Alabama.* Gamma-globulin levels were measurable in unconcentrated stimulated whole saliva and in parotid and submandibular secretions by an immunochemical precipitin method (see abstract #233). The concentrations varied within and among subjects and declined with continued stimulation of salivary flow. Thirty-one young male adults with normal erythrocyte sedimentation rates, A/G ratios, and thymol turbidity tests were divided on the basis of clinical and bite-wing radiographic examination into four subject groups: A, low and B, high caries rate with healthy gingiva, C, low and D, high caries rate with inflamed gingiva. The means and standard deviations are tabulated in micrograms

Subject Group	Parotid Fluid	Whole Saliva	Plasma
A (9)	11.6±6.88	11.9±3.31	2,226±330.6
B (7)	5.9±3.27	10.0±1.16	1,978±381.1
C (7)	6.1±1.75	12.5±3.15	2,327±472.2
D (8)	8.7±3.90	14.7±4.15	2,092±315.8
Total (31)	8.4±4.97	12.3±3.46	2,156±378.0

nitrogen per milliliter body fluid. Whole saliva and parotid concentrations were independent of plasma concentrations. The salivary fluid concentrations were positively correlated ($r = +0.458$, $P<0.01$). The mean whole-saliva level was reliably higher than the mean parotid concentration ($t = 4.98$, $P<0.01$). Gamma-globulin concentrations in saliva and parotid fluid did not differ significantly in oral health and disease

(variance analysis for parotid among the four groups: $F = 2.73$, $P > 0.05$). In another series, hypergammaglobulinemia was not necessarily associated with increased salivary levels.

138. Comparative Study of Submaxillary and Lacrimal Glands of the Rat.—*Theodore E. Bolder and John H. Manhold, Jr., Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey.* Striking similarities in composition and response to disease processes have been demonstrated for salivary and lacrimal glands. This study determined the composition and dry weight of lacrimal glands and compared some of the data with data obtained in a similar fashion for submaxillary glands. Lacrimal glands from 7 groups of untreated male albino rats were used (15, 30, 60, 90, 150, 300, and 500 days). A quantitative estimate was made of the relative areas in sections of left glands which were occupied by acinar and by intralobular duct tissue. An ocular grid divided into 66 squares was used. A total of 1,200 squares was counted. The number of squares in which half or more of the area was occupied by acini or ducts was counted in 3 specimens from each age group. Dry-weight determinations were made on the right gland of 27 rats from the last 6 ages. The relative proportion of acinar tissue to intralobular duct tissue remained fairly constant at all ages. The normal gland consisted of about 82 per cent acinar tissue and 3 per cent intralobular duct tissue. Lacrimal glands consisted of approximately 24 per cent more acinar tissue and 22 per cent less intralobular duct tissue than the submaxillary glands from rats of the same age. An average dry weight of 8 mg. (30 days) was increased to 50 mg. (60 days) and fell and fluctuated around 25 mg. at all other ages. The ratio of gland weight to body weight was 0.075 and 0.189, respectively, for lacrimal and submaxillary glands.

139. Transplants of Salivary Glands and Parotin.—*Harold S. Fleming, College of Dentistry, Howard University, Washington, D.C.* Salivary gland tissue was obtained from guinea-pig fetuses and transplanted to the brains of young adults. The techniques for brain transplantation have been reported elsewhere. A period of 3–5 days was allowed for the host animals to recover from the operations. The animals were then divided into 2 groups, with 1 group receiving semiweekly injections of $\frac{1}{2}$ cc. of normal saline and the other group receiving semiweekly injections of 0.75 mg. of parotin in $\frac{1}{2}$ cc. of normal saline. After the third injection, animals were killed intermittently following administration of this substance. A total of 10 injections was usually given. Recovered transfers from parotin-injected animals showed better vascularity of the transfers, with more patent blood vessels, than did the controls. The treated transfers were likewise more cellular, with a greater number of cells in acinar groups and ductal systems. More numerous mitotic figures were also located among the acinar cell groups and ductal epithelium. Within the ducts there appeared to be secretory products in both instances, but these were much more evident in the treated hosts. Greater numbers of the cells of the salivary gland transplants seemed to survive transplantation where parotin was administered. There also seemed to be a better acceptance of these grafts in parotin-treated animals, for antibody reactions, as manifested by chronic inflammatory cells and/or cytotoxic reactions to the transplants, were not remarkable. In addition, the so-called blood-graft barrier was better established with the homografts in treated animals.

140. Salivary Gland Ligation Extraction Wound Healing.—*George W. Wade and Harold S. Fleming, College of Dentistry, Howard University, Washington, D.C.* Salivary glands in 28 young adult male Wistar rats were ligated. Controls were 28 additional sham-operated rats. Thirty days following this operation an upper molar tooth was removed in each animal. Animals were sacrificed at intervals from the first to the thirty-fifth postextraction day. Jaw sections and certain organs were removed and prepared for histologic study. The differences observed were striking with regard to healing between the ligated and non-ligated animals and indicate that healing was

retarded in all the ligated animals. Secondary infections were more extensive and deeper, lasting for longer periods in the ligated rats. Epithelium did not begin to grow back over the surface in the ligated animals as rapidly as in the non-ligated ones. The covering epithelium was very thin in the ligated animals at the thirty-first and thirty-fifth days. It was three to four times thicker in the controls. Disturbances in alveolar bone were still more extensive in the ligated than in the non-ligated animals. Numerous chronic inflammatory cells were also found in association with the alveolar bone. The dermis presented an entirely different picture in the non-ligated animals, for in the ligated animals there were numerous blood vessels of varying sizes. Some showed hyperemia, with extravasation of red blood cells into the adjoining tissues.

XII. RADIOBIOLOGY AND ORAL BIOLOGY I. MARCH 24, AFTERNOON, SECTION D

141. RADIOCALCIUM TURNOVER IN SKULL SUTURES OF RHESUS MONKEY.—*P. K-J. Yen and J. H. Shaw, Harvard School of Dental Medicine, Boston, Massachusetts.* To determine the turnover of Ca^{45} in skull sutures, three young male rhesus monkeys were injected intravenously with 5–7.5 mc Ca^{45} as $CaCl_2$ in isotonic saline and sacrificed after 168 hours. Each bone segment containing a suture was removed from skulls with a dental separating saw. Appropriate samples were collected from suture and each adjacent bone. Ca^{45} radioactivity and total calcium were determined. A 100 per cent equilibration of tissue-specific activity and serum-specific activity occurred in soft tissues within 24 hours. After 168 hours the average percentages of equilibration were as follows: (A) Cranial bones: bregma, coronal, and sagittal sutures, 30 per cent; lambdoidal, 36 per cent; lambda, 40 per cent; whereas frontal, parietal, and occipital bones adjacent to these sutures were 12, 15, and 10 per cent, respectively; sphenoccipital synchondrosis, 50 per cent; sphenoethmoidal, 28 per cent. (B) Facial bones: frontonasal suture, 47 per cent; maxillo-premaxillary, 39 per cent, with 39 per cent on maxillary surface and 91 per cent on premaxilla; zygomatico-frontal, 52 per cent (same suture in temporal fossa, 36 per cent); zygomatico-maxillary, 36 per cent; zygomatic arch, 67 per cent, with 24 per cent on zygomatic and 88 per cent on temporal (lateral surface); maxillary tuberosity, 86 per cent; and pterygoid process, 30 per cent. (C) Norma basalis: medium suture of maxilla and palate, 42 and 20 per cent, respectively; anterior portion of glenoid fossa, 18 per cent; posterior, 35 per cent; occipital condyle, 53 per cent. The values were different at various locations along the same suture, indicating different stage of formation, proliferation, and calcification. Different turnover was also observed on both sides of certain sutures. This indicated that one bone may have more growth than the other.

142. RETENTION OF RADIOVANADIUM IN RAT MOLARS.—*Henry M. Leicester and Paul R. Thomassen, College of Physicians and Surgeons, San Francisco, California.* Thirty-two Osborne-Mendel rats at 2 days of age were injected intraperitoneally with 5.60 μg. of vanadium per kilogram of body weight, with radiovanadium 48 and 49 as tracer. These injections were continued every other day until the young were 30 days old. At this age the crowns of the third molars were fully calcified, and all molar teeth should contain vanadium. Three days later, 10 animals were sacrificed, and several select organs and tissues, including the molars and incisor teeth, were removed and dried in an oven at 60° C. for 48 hours. They were then ground to a fine powder and weighed. Each tissue was subjected to tracer analysis employing a gamma scintillator. Thirty, 60, and 90 days later, a group of from 6 to 8 rats was sacrificed, and the organs and tissues were removed and analyzed as described above. For the length of the experiment, vanadium appears to remain fairly constant in molar tooth structure. There is no apparent uptake in lymph nodes and thyroid. It is removed rather rapidly from such tissues as blood, heart, lung, striated muscle, intestine, and brain. It remains longer in liver, spleen, and salivary glands. Its primary sites of deposition seem to be the calcifying tissues, such as bone and tooth structure. The main source of removal from the body for vanadium appears to be the kidney.

143. Breaking Strength and Ash Analysis of X-Radiated Mouse Femurs.—*John A. Cameron and James L. Matthews, Baylor University College of Dentistry, Dallas, Texas.* Mice radiated in air with 300 r at 15 days of age in utero show an initial decrease in the rate of bone growth in both length and width. The size and growth rates of mandibles and femurs have been determined at weekly intervals up to 90 days. At the 30–60-day period the radiated bones attain the size of the control bones, and an apparent recovery from radiation is indicated. Histologically, the radiated bones appear to be at an earlier stage of development than the control bones. Bone strength and ash content were studied further to assess the quality of the radiated bone. Breaking strengths were determined by applying graded loads to the shaft of the bone. Breaking strength of radiated bones was 28 per cent less than that of control bones of comparable size. Analysis of bone ash calcium by the flame photometric method of Winer and Kuhn and flame photometric analysis of ash sodium were made. Although per cent calcium in the ash of radiated and control bones was not significantly different, the total ash of radiated bones was significantly less than the control. Sodium values of radiated and control bone ash were comparable in the 21–90-day bones. The sodium in the ash of radiated bones at 14 days was significantly higher than the control. Whether this is a radiation-induced phenomenon or is a characteristic of normal bone at an earlier stage remains to be demonstrated.

144. X-Ray Spectra after Transmission through Aluminum Penetrometers.—*Harry D. Spangenberg, Jr., and M. L. Pool, Ohio State University, Columbus.* The quality and the intensity of X-ray spectra after transmission through penetrometer steps have been measured by a scintillation spectrometer whose crystal is located at the position usually occupied by an X-ray film. Transmitted spectra were measured when 45-, 70-, and 90-kv. beams were unfiltered by added external filtration or were filtered by 0.5-mm. aluminum or by 0.5-mm. aluminum plus 0.375-mm. copper. For a given kilovoltage (e.g., 70 kv.) the shift in the energy of the transmitted peak intensity toward higher energies becomes progressively less (e.g., 21.1-, 16.2-, and 3.45-kv.) as the external filtration increases. When little or no external filtration is used, the shift in the energy of the transmitted peak intensity toward higher energies (e.g., 12.8-, 21.2-, and 25.8-kv.) becomes progressively greater as the kilovoltage increases from 45 to 90. When an external filter of aluminum plus copper is used, this shift reverses and becomes progressively less (e.g., 4.17-, 3.45-, and 3.00-kv.). The ratio of the peak intensity measured when no penetrometer is placed in the beam to that with the thickest penetrometer step in the beam (1) decreases as the external filtration increases, the kilovoltage remaining constant, and (2) decreases with increase in kilovoltage, the external filtration remaining constant. These results demonstrate that, in addition to the change in intensity because of attenuation by aluminum penetrometer steps through which the X-ray beam passes, there is also an accompanying shift in the peak energy of the transmitted X-rays by as much as 85 per cent.

145. Historadiographic and X-Ray Densitometric Study of Human Cementum.—*Narendar N. Soni and Grant Van Huysen, Indiana University School of Dentistry, Indianapolis.* Morphologic differences and variations in the mineralization of plano-parallel ground sections of human cementum were studied by means of microradiographs. Contact radiographs of the 20-μ-thick plano-parallel ground sections of cementum were obtained with a variable X-ray machine* equipped with a Machlett copper-target tube, operated at 20 kv. and 10 ma. and a target film distance of 10 cm. Microdensitometric tracings were obtained from these contact radiographs with a television microscope operating with a slit-width equivalent to 1 μ.[2] Microradiographs were obtained by making photomicrographs of the contact radiographs. In the microradiographs, two types of cementum were distinguishable, one the alacunar type (acellular) and the other the lacunar type (cellular). The structure of the latter resembled

* General Electric.

that of old bone. It contained lacunar elements in varying numbers. Historadiographs revealed bands with high X-ray absorption, separated by alternate bands with low X-ray absorption. These bands varied in thickness and showed a wavelike pattern, so that they often appeared oriented in slightly different directions with respect to each other. The degree of irregularity of these bands varied in different parts of the cementum. Generally, the alacunar cementum was more highly mineralized than the lacunar type. Microdensitometric tracings revealed an irregular and inconstant mineralization pattern in the different parts of the cementum.

146. The Morphologic Effects of Strontium 90 on the Major Salivary Glands of the Rat.—*F. Sobkowski, P. Dale, G. Casarett, L. Tuttle, and R. Baxter, Departments of Dental Research and Radiation Biology, University of Rochester, Rochester, New York.* Seventeen male rats, 8 months of age, obtained from the Rochester Colony of the Long-Evans Hooded Strain were divided into a control and an experimental group. The experimental group received a total dose of 160 mc of strontium 90 administered as a drinking solution in ten daily doses over a 2-week period. All animals were sacrificed 9 months later. Specimens of the parotid, submaxillary, and sublingual salivary glands were fixed in 10 per cent neutral formalin, imbedded in paraffin, sectioned at 7 μ, and stained with hematoxylin and eosin. The changes observed include degenerative alterations in the acinar and ductal cells, perivascular fibrosis, loss of patency of the microcirculatory apparatus, hyaline degeneration of the connective tissue stroma, focal necrosis, and fibrotic replacement of the acini. The submaxillary glands exhibited the greatest and the sublingual glands the least degree of histopathology, respectively. This observation does not necessarily imply a scale of relative radiosensitivity among the glands because the observed alteration may be dependent on anatomic relationships between the glands and the sites of deposition of the radioelement.

147. Eye Implants of Embryo Teeth in Scorbutic Guinea Pigs.—*Henry M. Goldman, Bernard S. Gould, and Joseph Stolman, Boston University School of Medicine.* The histologic bioassay technique to ascertain the state of scorbutus of the guinea pig has been well established. This method utilizes histologic preparations of transverse sections of the incisor tooth, from which measurements of the height of the odontoblastic layer of the pulp are made. It has been shown that the length of the odontoblasts varies with the intake of ascorbic acid. Using this method, guinea pigs weighing 250–300 gm. were placed on a scorbutigenic diet that resulted in the first signs of scurvy within 13–15 days. Embryonic tooth germs were implanted in the anterior chamber of the eye, and the animals were maintained for as long as 5 weeks, at which time the animals died of scurvy. Histologic examination of the incisor of the jaw revealed typical signs of scorbutus, whereas the implanted incisor showed normal development. Furthermore, embryonic bone implanted under similar conditions also revealed normal development. It would appear that embryonic tissue may not require an external source of ascorbic acid for its mesenchymal development.

148. Improved Version of the Contourator—an Instrument for Recording Changes of Mucosal Surface Contours.—*Lucian Szmyd, C. M. McCall, Jr., A. A. Brewer, and C. F. Schuessler, School of Aviation Medicine, USAF Aerospace Medical Center, Brooks AFB, Texas.* Significant improvements in the contourator have been developed which allow for greater accuracy, ease, and rapidity in recording topographic changes of the alveolar ridge. A pantographic apparatus has been constructed which enables the contourator to magnify the area of topographic changes in successive casts by a factor of 9. This is accomplished without linear distortion of the tracings. The contour arm is designed to operate through 360°. This design allows for an unobstructed approach to all undercut areas of the dental casts. Mounting of successive casts is accomplished by utilizing pre-keyed plates. The base upon which the dental casts are mounted can be moved in lateral, anteroposterior, and circular directions. These move-

ments are calibrated by means of micrometric scales, in order to permit precise location of selected areas on successive casts.

149. The Isolation and Identification of Oral Lipid Components.—*T. R. Dirksen, K. G. Ikels, and T. B. Weber, School of Aviation Medicine, USAF Aerospace Medical Center, Brooks AFB, Texas.* Further to characterize lipid components of dentin, carious material, and parotid secretion, alcohol-ether extracts of these samples were analyzed by paper and column chromatography. Chicle-stimulated saliva was collected with conventional parotid caps and frozen until used. Dentin obtained from roots of freshly extracted teeth was isolated, pulverized to 60 mesh, and stored at 4° C. Carious material was removed from freshly extracted teeth immediately preceding lipid extraction. Lipid material was separated from the samples by heating for 2 minutes at 50° C. in methanol. Anhydrous ether was then added, and the material was filtered through glass wool. The filtrate was washed with 0.85 per cent saline, evaporated to dryness, and reconstituted in either chloroform for paper chromatography or petroleum ether for column separation. Ascending, one-dimensional paper chromatograms were run on silicic acid-impregnated paper in solvent mixtures of *n*-heptane/diisobutyl ketone (86/6) for non-phosphatides and diisobutyl detone/glacial acetic acid/water (40/20/3) for phosphatides. To detect lipids, chromatograms were dipped in Rhodamine 6G and viewed by ultraviolet light. Column chromatography was conducted on micro-columns of silicic acid with elution by varying concentrations of diethyl ether in petroleum ether and methanol. Quantitative extraction of parotid secretion gave 1.1 mg/100 ml of alcohol-ether–soluble material. Dentin samples ranged from 1.2 to 1.5 mg/gm; carious material gave concentrations several times that amount. Cursory results indicated the presence of free fatty acids, monoglycerides, triglycerides, free cholesterol, cholesterol esters, and phosphatides in dentin and carious material.

150. The Chemistry of the Resorption of Diphenylhydantoin*-induced Collagen.—*J. C. Houck, Children's Hopsital and Georgetown University Medical Center, Washington, D.C.* Recent investigations in this laboratory have demonstrated that various components of connective tissue may be separated by sequential extraction in the cold. Employing 0.15 M NaCl, 0.5 M NaCl, and 0.5 M citrate buffer (pH 3.6), sequentially, the ground substance, neutral and citrate-soluble collagens may be extracted, and the insoluble collagen content of the tissue calculated. Using this technique, intraperitoneally administered sodium dilantin appears to decrease the neutral soluble collagen, increase the citrate-soluble collagen, and double the insoluble collagen content of rat skin. In rats previously subjected to 4 daily intraperitoneal doses of 25 mg. of sodium dilantin at various periods of time, alteration in the distribution and concentration of dermal collagen was noted. Within 2 weeks after conclusion of dilantin administration, the total collagen content of the skin had returned to normal, as did the dermal concentration of insoluble non-collagenous protein. The total hexosamine content of the tissue, however, still remained elevated. The pattern of changes in the distribution of the various collagens will be discussed in the light of collagen resorption in particular and collagenase activity in general.

151. Age, Gonadectomy, and Wound Healing in the Palatal Mucosa.—*Earl O. Butcher and Jules Klingsberg, Department of Anatomy, College of Dentistry, New York University.* Circular wounds, 1.5 mm. in diameter, were made with a trephine posterior to the second ruga in the palatal mucosa of rats, 22–850 days in age. A protective cylindrical shield around the trephine limited the depth. Epithelial surface regrowth was usually established by the sixth postoperative day, when an area including the wound was biopsied, histologically prepared, and stained either in Mallory's connective tissue, Weigert's resorcin fuchsin, PAS, or hematoxylin and eosin. Extent of wound healing was judged by examining the condition of the surface epithelium, the epithelial–connective tissue attachment, and the organization and regrowth of the con-

* Sodium Dilantin.

nective tissue fibers. Epithelial regrowth occurs over an extremely cellular connective tissue which is sharply delineated from the normal submucosa and in which there is a complete absence of both elastic and collagen fibers. There was generally little glycogen and no basement membrane in the epithelium over the wound. Epithelial surface cells were usually more normal in appearance than the basal cells. Wounds in animals 200 days of age or older heal less rapidly than those in 50-day-old animals. Aged animals have more defects in healing. Wound repair is similar in males and females of the same age. Ovariectomy had no effects, while healing was accelerated by castration.

152. Allergic Response of Oral Mucosa to Foreign Protein.—*Stephan E. Mergenhagen and Anthony A. Rizzo, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda, Maryland.* Allergic-type mechanisms may play an important role in the development of pathologic lesions of periodontal disease. To study one manifestation of the allergic response in oral tissue, the classical method of sensitizing a local site to foreign protein (Arthur phenomenon) was employed. New Zealand rabbits (2.0 kg.) were given 1.0-ml. injections of horse serum every other day subcutaneously in the intrascapular area. After 3, 5, and 6 sensitizing injections, groups of 5 animals were challenged intramucosally in an area including gingival and palatal mucosa. Another group of 12 rabbits was given subcutaneous injections of horse serum daily until skin sites became edematous and hemorrhagic. These animals were then challenged intramucosally with single or multiple injections of horse serum or horse serum with 0.1 per cent agar to keep the antigen localized in the mucosa. Animals sensitized with as few as 3 injections over a 6-day period showed an exaggerated inflammatory response to intramucosal challenge. Rabbits sensitized for longer periods and challenged intramucosally gave a lesion consistent with a true hypersensitivity reaction: marked edema, necrosis, and plasmacytosis. Non-sensitized control animals showed minimal response. Another manifestation of the allergic response in oral tissues was demonstrated by sensitizing a group of 9 rabbits to human tubercle bacilli in a complete Freund adjuvant. Seven weeks after sensitization, animals were challenged intramucosally with 0.01 mg. tuberculin purified protein derivative. After 72 hours, mucosal sites showed characteristics of a delayed hypersensitive response, including marked eosinophilia.

153. Observations of Condylar and Mandibular Movement during Function.—*I. M. Sheppard, Montefiore Hospital, New York City.* The pure condylar rotation found on articulators and used in some diagnostic and restorative techniques was investigated in 10 patients by means of rapid serial radiography. The bolus—a walnut meat—was chewed for 5 seconds, during which time 30 exposures on 14″ × 14″ film were made in lateral projection. Radiopaque markers were inserted in the upper and lower incisor regions and in the upper and lower molar areas, where metallic landmarks were not already present. Several condyle positions were traced, as was one mandibular outline. Tracing of representative lower incisor marker positions indicated that there was no constant path of opening or closing in chewing the walnut meat, that there was a tendency toward anteroposterior and posteroanterior shifts of the mandible. The irregular path of movement found resembled in general direction an arc drawn from an area in the ramus. An arc drawn from any of the condyle positions failed to resemble the general direction of functional closure.

XIII. DENTAL CARIES II. MARCH 24, EVENING, SECTION A

154. A Mathematical Theory of Early Enamel Caries.—*Stuart O. Zimmerman, University of Chicago.* An attempt to develop a theory of enamel solubility and early enamel caries has been made. Reasoning from solubility equilibrium studies of hydroxyapatite made by Levinskas suggested that the concentrations of calcium ion and monohydrogen phosphate ion might be the determining elements in the rate of solution of enamel. A model was set up in which the forward rate of solution of enamel

is considered to be constant and the backward rate a second-order kinetic term involving the product of the concentrations of calcium ion and monohydrogen phosphate ion. Hydrogen ion asserts its effect on the solubility rate by uniting reversibly with the monohydrogen phosphate to produce dihydrogen phosphate. When this model is applied to a situation of powdered enamel dissolving in buffered acid solution, the equations can be readily solved for the concentrations of calcium and total phosphate in solution as functions of time, provided that equilibrium is assumed to exist between the three forms of phosphate—monohydrogen phosphate, dihydrogen phosphate, and phosphoric acid. A comparison of the results of the theory with experimental solubility data in the literature was made. The data of Suess and Fosdick (*J. D. Res.*, **30**:177, 1951) agree rather well with the predictions of the theory over the range of pH 4.1–6.1. The data of Blackwell and Fosdick (*J. D. Res.*, **35**:210, 1956) do not agree quite so well over the same pH range because of the large change in surface area of enamel in their experiment, which is not accounted for in this model but which is accounted for in a later model not presented here. Even so, the agreement of the asymptotic, or equilibrium, values of the concentrations taken from their data is still fairly good. Extension of the model to a situation more closely akin to actual caries with diffusion into the enamel, varying hydrogen ion concentration, and differential permeation rates for the different phosphates is also discussed.

155. (Not presented.)

156. Yearly Intrapair Caries Differences between Monozygotic and Dizygotic Twins.—*Sidney B. Finn, Robert C. Caldwell, and Mary Grogan, University of Alabama School of Dentistry, Birmingham, Alabama.* This paper is a report of the dental caries findings of thirty-two pairs of monozygotic twins and twenty-nine pairs of like-sexed dizygotic twins over a 2-year period, consisting of three examinations exactly 1 year apart. The twins averaged approximately 10½ years of age at the beginning of the study. There was an average yearly increment of 1.26 DMF teeth for the monozygotic twins and 1.29 DMF teeth for the dizygotic twins, showing no statistically significant difference between the two groups. However, when one compares the intrapair DMF variation for the monozygotic and dizygotic twins, there is a statistically significant difference between the types of twins. The dizygotic twins had twice the intrapair difference as that found in the monozygotic twins. Averaging the three examinations, these differences were 1.14 DMF teeth and 2.83 DMF surfaces in the monozygotic twins, while for the dizygotic twins it averaged 2.39 DMF teeth and 4.85 DMF surfaces. The comparative intrapair differences between the number of DMF teeth and surfaces of the two types of twins was significant at the 0.001 level. The identical twins had an average yearly eruption of 2.53 new permanent teeth, while the fraternal twins had an average eruption of 2.51 new permanent teeth, both types of twins having the same number of newly erupting teeth per year at these age levels. If one considers the intrapair variance in number of erupted teeth at each of the three examinations, one finds a statistically significant difference between the two types of twins. Among the identical twins it was 0.94 erupted teeth per year, while the fraternal twins had a variance of 2.27 erupted teeth per year. This difference could occur by chance in less than one in 0.001. The intrapair variance in number of DMF teeth coincides quite nicely with the variance in the number of erupted permanent teeth in each group of twins. Since environmental factors are fairly constant between each twin pair as determined by a dietary analysis and home surroundings, the differences in intrapair dental caries between the two types of twins can be considered as being affected by the intrapair difference in the number of erupted teeth. Since the length of exposure to the oral environment is an important variable in the production of dental caries, the eruption time of teeth in each individual is an important hereditary variable in relation to dental caries. This paper presents some evidence of this fact. Monozygosity was established by comparing fourteen physical features and by studying the following blood types: A, B, O, and Rhesus, as well as the MNS group, the P group, the Lewis

(a), the Kell, the Lutheran (a), the Duffy (a), the Gm (a), and the Hp groups. Any discordance in any of the blood types would rule out monozygosity. There was complete concordance in the twins listed as being monozygotic.

157. Effect of Added Water on the Caries Potential of Experimental Diets in the Hamster.—*J. F. Volker and C. E. Klapper, University of Alabama Medical Center, Birmingham, Alabama.* It is a consistent observation that desalivated hamsters develop severe experimental caries when fed diets containing a high content of sugars and starches. The present study was undertaken to determine the extent to which the cariogenic potential of such diets is altered by the addition of water. One hundred and sixty hamsters were desalivated on approximately their twenty-fifth day. Subsequently, they were divided into three major groups. Each group was maintained on different, but rather similar, cariogenic diets for periods up to 105 days. Within each group, subgroups received the diet as a powder and in thick and thin slurry forms. The thick slurry had 6 oz. of diet in 100 cc. of water, and the thin slurry had 4 oz. of diet in 250 cc. of water. Selected groups were sacrificed on the 61st, 78th, and 105th day and scored for caries. Regardless of the diet ingested, animals maintained on the thin slurry had less than one-third as much caries as those receiving the dry-powder form. The caries scores of the thick-slurry and thin-slurry groups were quite comparable. These findings illustrate the importance of diet fluidity as it relates to caries potential. They suggest the need of further study to determine the minimum fluidity that will reduce diet cariogenicity.

158. Importance of Genetic Strain and Tooth Age in Caries Studies.—*Kenneth O. Madsen and Eleanor J. Edmonds, University of Texas Dental Branch, Houston, Texas.* Cotton rat stock animals were inbred to establish several strains. When all strains were fed a cariogenic diet, six strains were found almost uniformly caries-susceptible. Their caries scores at the end of 98 days were twice as great as the scores found in one resistant strain. A re-evaluation of data previously reported (*Fed. Proc.*, **19**:322, 1960, abstr.) was made on the basis of the caries susceptibility of the rats used to obtain the data. When two varieties of rice hulls were fed as 15 per cent of the cariogenic diet, both were equally anticariogenic in the susceptible rats; only one variety reduced caries in the resistant rats. New information was also found in another study when the data from susceptible rats were evaluated separately. These rats were fed rice hulls at the 25 per cent level for various time intervals during the experimental period. Caries scores in the first and second molars were greatly decreased by feeding hulls from 18 to 28 days of age; but caries in these molars was not decreased by feeding hulls beyond the twenty-eighth day. The third molars, which erupted later, were protected when the feedings of hulls overlapped the period of eruption into the oral cavity. In all three molars no protection was demonstrable beyond about 18 days after eruption. Protection of the third molar beyond the eighteenth day was found, however, in another study in which hulls were fed (15 per cent level) for a prolonged period.

159. The Cariostatic Effect of Some Antibacterial Inhibitors.—*H. R. Muhlemann, R. Meyer, K. G. Konig, and T. M. Marthaler, Dental Institute, University of Zurich, Switzerland.* Antibacterial inhibitors have been re-evaluated in animal caries experiments under identical and comparable conditions. Using the procedure of incomplete block balances lattice design, Osborne-Mendel rats were assigned in 3 independent experiments to a total of 75 treatments. The rats, housed 3 per cage, were administered diet 580 from day 25 to day 40. Molar fissure lesions were diagnosed microscopically and scored as described previously (*Dtsch. Zahn-, Mund- u. Kieferheilk.*, **29**:99, 1958). The average number of initial dental lesions in 12 rats per treatment will be presented for 53 out of the 72 substances studied. Experiment I: Sodium fluoride, iodoacetic acid, penicillin, tetracycline, chlortetracycline, chloramphenicol, dihydrostreptomycin, nystatin, amphotericin B, usnic acid, sulfanilamide, sulfathia-

zole, sodium chlorophyll, methyl violet, sodium dehydroacetate sodium-*N*-lauroylsarcosinate. Experiment II: Sodium fluoride, iodoacetic acid, penicillin, oleandomycin, erythromycin, novobiocin, bacitracin, dodecyldimethyl-(phenoxy ethyl) ammonium bromide, benzalkonium chloride, phenylmercuric borate, dequalinium chloride, dequalinium fluoride, nitro-furaldehyde semicarbazone, acriflavine, bis (methyl amino chinolyl carbamid), *p*-(amino methyl) benzene sulfonamide, pyridoxine, nicotinamide, isonicotinic acid hydrazide, protamine, B-hydroxyquinoline. Experiment III: Sodium fluoride, iodoacetic acid, penicillin, demethyl-chlortetracycline, Thiostrepton, Ethoduomeen di-hydrochloride, Ethoduomeen di-hydrofluoride, sodium fluoride, flavofungin, griseofulvin, sodium metabisulfite, potassium rhodanate, orotic acid, phenanthroline, naringenin.

160. Studies on Rehardening of Artificially Softened Enamel.—*T. Koulourides, H. Cueto, J. Reed, M. S. Kim, and W. Pigman, University of Alabama Medical Center, Department of Biochemistry and New York Medical College, Department of Biochemistry.* Tooth surfaces softened in acetate buffer (0.001 M, pH 5.50) were exposed to solutions supersaturated with respect to calcium and phosphate ions. The enamel hardness was determined initially, after softening and after treatment by the rehardening solutions. The solutions used for rehardening contained 1.0–3.0 mM/l of calcium ions with sufficient phosphate to give a molar ratio of Ca/P of 1.67; often the solutions contained 0.05–0.35 mM/l of fluoride ions. All work was done at 37° C. The optimal initial pH for rehardening was found to be that at which incipient precipitation occurred as judged by examination of the Tyndall effect. It was inversely related to the initial calcium concentration and ranged from pH 6.8 for solutions containing 3 mM. calcium to 8.7 for solutions containing 0.5 mM. calcium per liter. The pH decreased during the rehardening process, and rehardening was found to occur only when the pH was greater than 6.4. With 0.05 mM. fluoride present, the pH decreased to about 5.5 in 2 hours, whereas in its absence the pH was 6.2–6.4 for solutions containing 1.5–2 mM/l of calcium. Under optimal conditions with fluoride present, complete rehardening occurred in about 3.5 hours, with one change of solution after 2 hours. Ground enamel and intact enamel could be superhardened about 10 per cent after such treatments. The effect of rehardening solutions on caries attack in the artificial mouth was tested by preparing media supersaturated with respect to calcium phosphate containing 7 ppm fluoride. Five days exposure of 6 teeth to softening in artificial mouth using this media did not change their hardness significantly. The teeth exposed to the control medium for the same time were softened beyond the limits of hardness measurement.

161. Residual Cariostatic Action of Phosphate in Hamster Diets.—*R. S. Harris, A. E. Nizel, and J. M. Parker, Department of Nutrition, Food Science and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts.* A group of 30 weanling hamsters was fed caries-producing diet MIT #10 (corn 47, sucrose 14, non-fat milk powder 20, plastic creem 10, alfalfa 6, liver concentrate 2, and NaCl 1). A similar group of 30 hamsters was given the same diet supplemented with 5.43 per cent Na_2HPO_4. The P contents of these two diets were 0.4 and 1.6 per cent, respectively. After 70 days, both groups showed very mild caries development. Both groups were then transferred to Keyes CPD 15-30 caries-producing diet for 70 days. The molars were then examined under binocular microscope for extent of caries development and occlusal attrition. Of the hamsters which had received two caries-producing diets in sequence, 75 per cent showed dental caries, while only 24 per cent of those which had received the $NaHPO_4$-supplemented diet followed by the Keyes diet showed caries. Morphologic change was noted in 27 per cent of $NaHPO_4$-fed hamsters, and no change in the control group. It is evident that the phosphate-supplemented diet fed during the first 70 days altered the teeth of the hamsters, making them more resistant to the cariogenic activity of the diet fed during the following 70 days.

162. DENTAL CARIES IN THE PROGENY OF ACIDOTIC PREGNANT RATS.—*C. T. G. King, M. L. Kunde, and F. J. McClure, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda, Maryland.* Fifty female Sprague-Dawley rats from the National Institutes of Health stock colony, 60–70 days of age and raised on Purina Lab Chow, were used in this study. At different periods during pregnancy, these animals were rendered acidotic by exposure to an atmosphere of 30 per cent CO_2 in 70 per cent O_2. This treatment lowered the blood pH to 6.86, as compared with a normal pH of 7.4. The litters from treated and non-treated females were reduced to six in number and, after 21 days of lactation, were fed a cariogenic skim-milk-powder diet. After a period of 65 ± 5 days these rats were sacrificed and the lower molar teeth scored for caries. There were 75 rats whose mothers had been exposed to the CO_2 atmosphere during the seventh day of gestation for 30 minutes and on the eighth and ninth days for 10 minutes. Of these, 97.4 per cent were carious and had an average severity score of 19 ± 1.8. Among 67 rats from non-treated females, 83.6 per cent developed caries, with an average score of 9.25 ± 1.23. The majority of the carious lesions developed on buccal surfaces and were typical of this type of diet. Acidosis after the fourteenth day of pregnancy did not alter the caries susceptibility of the offspring on the cariogenic diet. This treatment also did not induce caries in rats maintained on a normal stock laboratory diet.

163. FURTHER STUDIES IN THE INHIBITION OF EXPERIMENTAL CARIES BY SODIUM METABISULFITE.—*Harold V. Jordan, Robert J. Fitzgerald, and Norma D. Berger, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda, Maryland.* In an attempt further to clarify the cariostatic action of sodium metabisulfite, other sulfur-containing compounds, including sulfites and sulfates, were tested for their comparative effects on experimental caries in rats. The compounds were blended into a coarse corn-particle diet (NIDR 585) and fed for 90 days to experimental groups composed of 24 Sprague-Dawley female rats. Dietary concentrations of 0.04 M sodium sulfite, 0.02 M sodium metabisulfite, 0.02 M sodium hydrosulfite, and 0.04 M sodium bisulfite inhibited caries development by 89, 84, 80, and 74 per cent, respectively, based on the caries scores. Sodium sulfate (0.04 M) had no apparent effect on caries. The feeding of 0.04 M sodium bisulfate was accompanied by a 24 per cent reduction in the caries score. This was not statistically significant. In the molars of animals receiving bisulfate the occlusal surfaces showed extensive wear, and there was a loss of enamel from the lingual surfaces suggestive of erosion. Vitamin supplementation of metabisulfite-containing diet 585 was also tested because of the known deleterious effect of sulfites on vitamins, particularly thiamin. The vitamin supplement contained B vitamins, menadione, ascorbic acid, and the fat-soluble vitamins, A, D, and E. Also included in the experiment were parallel groups in which the diets were autoclaved for 30 minutes before adding vitamins. Vitamin supplementation of these diets had little or no effect per se on weight gains or caries scores.

164. A HYPOTHESIS ON IMMUNE TOLERANCE AS A MECHANISM OPERATING IN HAMSTER CARIES.—*Norman S. Simmons, William H. Hildemann, and Reidar F. Sognnaes, University of California Schools of Medicine and Dentistry, Los Angeles.* Observations on the transmissibility of experimental caries in hamsters have implicated a particular strain of cariogenic organisms as the infectious agent (*Arch. Oral Biol.*, **1**:304, 1960). Details of the nature of the infectivity, as well as the marked variability in caries activity between hamster colonies reported by many investigators, invite the consideration, however, that an additional phenomenon on "immune tolerance" (*Proc. Roy. Soc.*, **146**:1, 1956) may be involved. This state of the defense mechanism of the host, acquired by exposure to exogenous antigen (e.g., bacteria) during a neonatal period of immunological immaturity, influences the immune mechanism so as to preclude appropriate antibody response to further antigenic challenges in the adult. Al-

though this neonatal period of immunological immaturity varies among species, it is particularly prolonged in the hamster, lasting 1 to several weeks. There is, thus, ample time for such a mechanism to operate during the exposure of litters to strains of organisms characteristic of the maternal flora. The hypothesis is therefore offered that (1) high susceptibility to caries may be a consequence of neonatal exposure to cariogenic strains of organisms of the maternal flora, with the development of immune tolerance and failure of adult defenses; and (2) low susceptibility may result from absence of neonatal contact with these cariogenic organisms, permitting full antibody production and protection of the adult against infection at any time; conversely, neonatal contact with anticariogenic flora would allow the full growth of these organisms in the adult, preventing subsequent infection by cariogenic strains.

165. Dental Caries in Hamsters Induced by Transfer of Cariogenic Plaque.—*Paul H. Keyes and Robert J. Fitzgerald, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda, Maryland.* The development of dental caries in albino hamsters following the transfer of plaque material from teeth of animals with caries has been studied. Newly weaned animals were anesthetized with Nembutal while the plaque was introduced into the interproximal space and lingual gingival crevice of upper right first and second molars. Some animals infected by this method have shown plaque growth and early signs of caries within 4 days, especially in the upper right second molars. Almost all animals infected by this method to date have shown moderate to extensive cavitation 42–50 days after inoculations, and the disease spreads to other teeth. No caries develops in uninoculated controls. With plaque from animals previously inoculated with streptomycin-resistant cariogenic streptococci (*J.A.D.A.*, **61**:9, 1960), recovery and identification of the mutant strain have been excellent. This technique, in conjunction with the caries-test diet previously described (*J. D. Res.*, **38**:525, 1959), has induced caries somewhat more rapidly than other methods: viz., pure cultures of cariogenic streptococci, feeding of feces from infected animals, exposure to hamsters with caries, and exposure to desalivated hamsters with caries. The "labeled"-plaque inoculation technique offers particular promise in the study of the mode of action of potential anticaries agents. After establishment of caries activity in the first and second molars, treatment with the test substances can be instituted before the third molar erupts. The effects on initiation and progression of lesions and the fate of the cariogenic microflora may thus be studied concurrently.

166. Hereditary Limitations on the Infectious and Transmissible Nature of Experimental Dental Caries.—*S. Rosen, H. R. Hunt, and C. A. Hoppert, Michigan State University, East Lansing.* This study confirms that dental caries is an infectious and transmissible disease in albino rats. The transmission of this disease, however, was significantly demonstrated only with rats which were genetically susceptible but not with rats which were genetically resistant. After depressing the penicillin-sensitive flora and reinoculating with feces from either caries-susceptible or caries-resistant rats, there was a significant increase in caries activity in caries-susceptible but not in caries-resistant rats. Concepts concerning the importance of heredity in the causation of caries held prior to the demonstration of the infectious and transmissible nature of dental caries, therefore, should not be altered.

XIV. CALCIFICATION. MARCH 24, EVENING, SECTION B

167. Effect of Bovine Submaxillary Mucoid on Ultrafiltration of Calcium.—*S. Wah Leung and Frank J. Draus, University of Pittsburgh School of Dentistry, Pittsburgh, Pennsylvania.* As an approach to the problem of the relationship of the organic matrix to calculus formation, we had previously studied the effect of a crude bovine submaxillary gland extract on the ultrafiltration of calcium, using the Toribara ultrafiltration chamber (*J. D. Res.*, **39**:731, 1960). The work has now been extended to include the study of a purified mucoid, isolated from the crude glandular

extract, on calcium ultrafiltration. The method of purification and the properties of the mucoid have been previously described (*Arch. Oral Biol.*, **3**:35, 1960). The effect of three variables on the ultrafiltration was investigated: pH, mucoid concentration, and calcium concentration. The pH effects were studied in solutions having an extract concentration of 1 per cent and a calcum concentration of about 6.5 mM/l, prepared in Tris buffer over a range of pH 3–10. The results showed a steady increase in the amount of calcium retained by the mucoid as the pH was raised. There was little retention up to pH 6, but above this the amount retained increased rapidly. When the pH was kept constant at either 6, 7, 8, or 9 and the mucoid concentration increased from 0.25 to 1.25 per cent, a direct relationship between mucoid concentration and calcium retention was demonstrated. At a constant pH of 9 and a mucoid concentration of 1 per cent, the amount of calcium retained increased steadily as the calcium concentration was raised until, at a calcium concentration of approximately 10 mM/l, the amount of calcium retained reached a maximum and remained unchanged with further increases in calcium concentration. At this point the amount of calcium retained was equal to 0.35 mM Ca/gm mucoid. Assuming that the mucoid has a molecular weight of approximately 91,000, this would mean that 32 calcium ions were bound to each molecule of mucoid.

168. AUTORADIOGRAPHIC STUDIES ON THE CALCIFICATION OF ENAMEL.—*Edward J. Reith, New York University, and Val F. Cotty, Bristol-Myers Company.* The route whereby calcium enters enamel during amelogenesis has been studied by means of autoradiography. Attention has been directed toward the calcification which occurs early, that is, almost immediately after matrix formation, and also that which occurs during the early phases of maturation in incisor teeth of rats. Pulp cavities of experimental teeth were occluded with a zinc oxide–eugenol mixture. Fillings were placed at the basal extremity of the pulp cavity and more incisally. Animals were then injected with calcium-45. After suitable periods, teeth were removed, ground, and autoradiographed. The unoperated tooth served as a control. Results showed that the pattern of calcium deposition was similar in test and control teeth regardless of where the filling was placed. Occlusion of pulp in test animals was indicated by observation and absence of radioactive calcium uptake in the dentin distal to the filling. We conclude, therefore, that the route whereby calcium enters the enamel during amelogenesis is via the enamel organ and not via the pulp.

169. INFRARED ABSORPTION SPECTROSCOPY OF CALCIFIED TISSUES.—*William H. Emerson, Tufts University School of Dental Medicine, Boston, Massachusetts.* Infrared absorption spectra of powdered enamel, dentin, bone, and supragingival calculus have been obtained in the region between 5,000 and 650 cm^{-1} using KBr as a suspending medium. These spectra have been compared with those of $CaCO_2$, hydroxyapatite, and mixtures of these compounds in proportions found in biological tissues. Spectra have also been obtained from samples of hydroxyapatite treated with weak solutions of $NaHCO_3$. Comparison of the spectra with special attention to the carbonate-absorption regions at 1,400, 870, and 710 cm^{-1} reveals the following: All of the biological materials have essentially similar spectra with respect to carbonate and phosphate. The band at 1,400 cm^{-1} is consistently a doublet, and the band at 710 cm^{-1} is always absent. Apatite treated with sodium bicarbonate solution gives a spectrum which closely resembles that of calcified tissues. Preliminary studies indicate that the spectrum of hard tissues is consistent with that of carbonate "adsorbed" on crystal surfaces. When enamel was suspended in KBr containing KCNS, the ratio

$$\frac{A(CO_3^{=})\,870\ cm^{-1}}{A(KCNS)\,2{,}100\ cm^{-1}}$$

was related to the concentration of $CO_3^{=}$. It is thus possible to use infrared methods for microestimation of $CO_3^{=}$ in calcified tissues using an internal standard.

170. On the Three-dimensional Morphology of Crystallites in Developing Deciduous Human Enamel.—*Erling Johansen and Harold F. Parks, Departments of Dental Research and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, New York.* In ultrathin sections and in triturated preparations of developing deciduous human enamel studied with the electron microscope, apatite crystallites were seen to present long, thin profiles somewhat suggestive of a needlelike shape, broad profiles indicative of a platelike shape, and profiles intermediate between these two extremes. With a stereoscopic technique similar to that previously described (*J. Biophys. Biochem. Cytol.*, **7**:743, 1960), two images of the same crystallites were recorded from different angles. The crystallites that were properly oriented exhibited a profile of different width in each of the two views. On the basis of this observation it is concluded that the crystallites of developing deciduous human enamel are platelike structures. This finding will be discussed in relation to relevant literature.

171. Oriented Precipitation of Hydroxyapatite and Other Inorganic Salts in Fibrous Matrix.—*Bernard N. Bachra, Otto R. Trautz, and Aldo R. Conetta, New York University, New York.* Electron microscope, X-ray diffraction, and crystal-optical studies of many vertebrate calcified tissues demonstrate that preferential orientation occurs of both fibrous matrix and the mineral deposited in it. It is generally assumed that the fibrous matrix is formed prior to its calcification and that the orientation of the mineral occurs during its deposition. Hydroxyapatite, strontium apatite, and thallous phosphate were orientedly precipitated in collageneous tendons (non-calcified turkey-leg tendon, native rat-tail tendon, bovine Achilles tendon) and in squid pens (β-chitin). Some experiments with artificial fibers did not result in oriented precipitation of these salts. In some instances we observe orientation of coarser crystallites of thallous phosphate precipitated on rat hair and surgical silk, while a fine-grained hydroxyapatite showed no orientations. Preliminary results indicate that blocking of free ϵ-amino, carboxyl, or hydroxyl groups or deguanidation of native rat-tail tendon does not impair its ability to orient the precipitates. A theoretical discussion is given of these results and of other experiments reported in the literature. The conclusion is drawn that, at present, the evidence is still insufficient to decide whether the orientation of the mineral crystals in calcified tissues is due to mechanical or to epitactic factors or both.

172. The Separation of the Mineral Phase in Mineralized Tissues.—*Racquel R. Zapanta and Otto R. Trautz, New York University, New York.* The study of the structure and chemical composition of the mineral phase in mineralized and other biological tissues requires a method of liberating the mineral phase with a minimum of structural changes, so that its behavior under varying conditions can be examined without the interference of the organic tissue components. Below 300° C., any structural change must occur by way of solution and reprecipitation and may possibly involve a change in chemical composition. Our amorphous calcium carbonate phosphate precipitate can serve as a sensitive test sample for demonstrating the effect of the separation method on the structure of the mineral phase. Even a slight crystallization is readily revealed by X-ray diffraction. Results: The older methods use KOH. Heating with a 5 per cent solution in glycerol or ethylene glycol (6–8 hours at 120°–130° C.) produced poorly crystallized apatite with short *a*-axis and slightly longer *c*-axis. We consider it a carbonate apatite. Aqueous 60 per cent KOH gave OH apatite. Secondary phosphate is transformed into apatite, excess calcium carbonate into $Ca(OH)_2$, and magnesium carbonate into $Mg(OH)_2$. More recently, boiling with ethylene diamine (B.P. 118° C.) has been employed. This is the safest separation method, as it best preserves the original state of the mineral. Even after 3 days' treatment, we could not detect any structural change in our amorphous precipitates or in amorphous biological concretions consisting of calcium and magnesium carbonate and phosphate. Pure $CaHPO_4$ $CaCO_3$, and $MgCO_3$ reagents remain unchanged.

173. EFFECT OF CRYSTAL PERFECTION ON COMPARATIVE UPTAKE OF Sr AND Ca BY CALCIFIED TISSUES.—*Aaron S. Posner, American Dental Association Research Division, National Bureau of Standards, and Robert C. Likins, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland.* Rats were injected interperitoneally with Sr^{89} and Ca^{45} and sacrificed at the end of 1 hour. The comparative deposition of Sr^{89} and Ca^{45} was determined in the ends and shafts of the tibia and in the incisor and molar dentin and enamel. The Sr^{89}/Ca^{45} ratio ranged from 1.00 in the tibia shaft to 0.76 in the incisor and molar enamel samples. X-ray diffraction line-broadening analysis of the specimens showed that the discrimination against Sr relative to Ca increased with an increase in crystal size and perfection. This is similar to the findings in an earlier report on the comparative uptake of Sr and Ca by a series of synthetic hydroxyapatites ranging in crystal perfection (*J. Biol. Chem.*, **235**:2152, 1960).

174. IN VIVO STUDIES ON THE ROLE OF THE ORGANIC MATRIX IN CALCIFICATION.—*George R. Martin, Anthony A. Rizzo, Stephan E. Mergenhagen, Doane N. Wright, and David B. Scott, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda, Maryland.* The role of the collagenous matrix of calcifying tissues in initiating and regulating the deposition of hydroxyapatite is at present under investigation in several laboratories. Our recent studies indicate that a normally non-mineralizing tissue (rat-tail tendon) will calcify when implanted in the peritoneal cavity of rats and rabbits. Electron-microscopic examination and histochemical studies suggest that mineralization proceeds in a manner similar to that previously observed in avian leg tendons, which do calcify normally in situ. Purified collagen reconstituted in 0.16 M NaCl at pH 7.0 does not mineralize when implanted. The addition of phosphate and in some cases alkaline phosphatase to collagen solutions during reconstitution produces a calcifiable matrix. Mineral deposited on these matrixes is observed as needle-shaped crystals on and within the fibrils. By limited area electron diffraction it can be demonstrated that the crystals are hydroxyapatite oriented parallel to the fibril axis. Our studies suggest that collagen fibrils regulate the positioning and habit of crystals but do not initiate the calcification process.

175. BONE INDUCTION ACROSS A MILLIPORE FILTER IN VIVO.—*Paul Goldhaber, Harvard School of Dental Medicine, Cambridge.* Further studies on the problem of bone induction have recently revealed that homograft bone, implanted subcutaneously within millipore diffusion chambers, could elicit new bone formation on the host side of the filter. This finding offered a means of testing immunologically whether such new bone was truly derived from host tissue or was merely the result of migration of bone-forming cells from the homograft across unseen breaks in the filter. Young male Swiss mice (Webster strain) were immunized to tissues of C57 bl/6 mice by three separate subcutaneous implantations, at 2-week intervals, of spleen fragments from adults of the latter strain. Following this 6-week immunization period, a diffusion chamber containing half a 1–2-day-old C57 bl/6 calvarium was implanted subcutaneously into each host, while the opposite half of the calvarium was implanted at some distance from the chamber as a "free-implant" control. Histologic examination of 14 intact chambers recovered from immunized mice sacrificed at 2, 3, and 4 months revealed patches of new, vital bone free of any homograft reaction on the host side of the filter in 12 animals, whereas the control homograft bone implanted freely was uniformly found to be dead, surrounded by inflammatory cells, and showed little, if any, attempt at new bone formation. These findings suggest that the new, vital bone found on the host side of diffusion chambers was derived from host tissue in response to a diffusible osteogenic inductor coming from the new homograft bone laid down on the inner aspect of the chamber filter.

176. Calcium and Phosphorus in Cementum and Root Dentin.—*Knut Selvig, Eastman Dental Dispensary, Rochester, New York.* Cementum and dentin samples from human teeth of various age groups were analyzed for total Ca + Mg and for P. Serial cross-sections of the teeth were made at 100 μ. From each tooth three sections were selected for chemical analysis, representing the apical, middle, and cervical areas of the root. Under polarized light, at 40 or 100 times magnification, the cementum was separated from the dentin. Most samples obtained were in the range of 100–200 μg. The samples were dissolved in 1 N hydrochloric acid. Total Ca + Mg was determined by titration with EDTA, using Eriochrome Black T as a color indicator. Phosphorus was determined spectrophotometrically. The results were expressed as percentage of dry weight. The values obtained for Ca + Mg and P in cementum were in all cases lower than those in the dentin of corresponding age group and root area. In apical dentin, and also in apical cementum, a tendency toward an increase in Ca + Mg and in P in old teeth was found. The ratio (Ca + Mg)/P in cementum and dentin did not change much with age but was in all cases higher in the apical than in the cervical area. The results indicate that changes take place in the mineral content of apical cementum with increasing age and that the changes are in the same direction as those occurring in dentin.

177. The Effect of Heat on the Carbonate Content of Dentin.—*Lorentz G. Nordback, Erling Johansen, and Harold F. Parks, Departments of Dental Research and Anatomy, University of Rochester, Rochester, New York.* It is generally recognized that the carbonate content of calcified tissues is influenced by the method of preparation of the samples. For instance, incineration at temperatures above 600° C. causes an almost quantitative removal of carbonate. In our current investigation on the inorganic phase of dentin it was of interest to establish the actual relationship between temperature and carbonate loss. Pure samples of pooled, powdered dentin prepared from the roots of freshly extracted teeth were dried for 24 hours at a temperature of 100° C., weighed, and exposed for 24 hours to constant temperatures varying from 200° to 800° C. Carbonate was analyzed as CO_2, using Sobel's modification of the van Slyke–Folch manometric method, with MacFadyen's reagents and factors. The carbonate content of the samples decreased with increasing temperature of incineration as follows: 100° C., 3.55 per cent; 400° C., 2.5 per cent; 600° C., 0.75 per cent; 700° C. and 800° C., traces. Electron micrographs will be presented to illustrate changes resulting from the heat treatment.

178. Solution Rate of Hydroxyapatite in Acetate Buffers Containing Low Concentrations of Foreign Ions.—*R. L. Speirs, M. Spinelli, and F. Brudevold, Forsyth Dental Infirmary, Boston, Massachusetts.* The solution rate of synthetic hydroxyapatite was studied in 0.2 M acetate buffers, pH 4.2, 5.2, and 6.0, containing foreign ions in concentrations of 0.1 mM or less. The solution rate was determined by estimating the weight loss of the solid and the release of Ca and P after brief periods (1–20 minutes) to exposure to acid. The ions studied included F^- (sodium), Zn^{2+} (acetate), Pb^{2+} (acetate), Mg^{2+} (acetate), $Mo_7O_{24}^{6-}$ (ammonium), VO_3^- (ammonium), SeO_3^{2-} (selenious acid), and, as a control, NH_4^+ (chloride). Of these, only F, Zn, and Pb significantly reduced the solution rate of hydroxyapatite. This effect was evident even after only 3 minutes. In an attempt to explain the action of these ions, the hydroxyapatite residues from control and experimental tests with F, Zn, and Pb were recycled in fresh buffer. The reduced solubility of the experimental residues suggests that a rapid uptake of these ions takes place during the first exposure to acid. Additional mechanisms by which these ions produce their effects on solution rate have been studied. The results with F confirm the observation made by Manly and Harrington (*J. D. Res.*, **38**:910, 1959), that 1 ppm F in acid buffer depressed the solution rate of powdered enamel.

179. CALCIFICATION IN ARTHROPODS.—*Leo Schatz and Gerrit Bevelander, New York University College of Dentistry, New York.* The decapod crustacean *Pagurus pollicaris* Say bears an exoskeleton resembling dentin. The skeleton may be sectioned without prior decalcification and includes both calcified and non-calcified zones. Preliminary studies reveal the presence of chitin in combination with a tightly bound, as well as a loosely bound, protein moiety. In regions which will calcify, histochemical and microchemical tests reveal a Millon-positive substance which reacts like tyrosine or one of its derivatives (e.g., tyramine, hydroxytyramine). The Millon-positive material is resistant to prolonged extraction with borate buffer at pH 9.2 but is extracted, along with tightly bound protein, by 24-hour treatment with 5 per cent NaOH at room temperature. Preliminary isoelectric point determinations, by a modification of Loeb's methods, indicate a pI of 4.2–4.8 for the Millon-positive zones. These zones also exhibit great cation-binding capacities, even after treatment with dilute HCl to remove preformed ions. Ion binding was demonstrated for the alkaline earths and certain transition and amphoteric metals. Variations in binding capacity related to ionic radius and charge remain to be verified by semiquantitative methods utilizing radio-isotopes. The inorganic portion of the skeleton seems to be almost entirely calcium carbonate, with minute traces of phosphate and the other ions and radicals found in sea water. Parts of the skeleton in which $CaCO_3$ crystals form, become non-metachromatic (i.e., unstained), while adjacent non-calcified zones remain metachromatic when treated with toluidine blue at pH 6.0. Studies are under way to determine the direction of crystal orientation with respect to the orientation of organic matrix micelles. Calcosphaerites appear in the epicuticle, while in endocuticle crystals with angles approximating those observed in calcite have been noted.

XV. PERIODONTOLOGY. MARCH 24, EVENING, SECTION C

180. MEASUREMENT OF THE STRENGTH OF THE ATTACHED EPITHELIAL CUFF.—*Gilbert J. Parfitt, University of Alabama School of Dentistry, Birmingham.* There is still a controversy as to whether the deeper epithelium surrounding the tooth is, or is not, physically attached to the tooth surface. Each proponent gives importance to the procedure of inserting a thin strip into the crevice, which, it is claimed, passes without hindrance to the bottom of the epithelial cuff or is arrested halfway down by an epithelial attachment, further insertion causing tissue destruction. Sensitive extensometer micrometers (electromagnetic) were used to sense motion and position of a 1.0×0.1-mm. steel strip introduced into the crevice, with the force applied to it at the same time. Both force and movement were recorded simultaneously on an X-Y recorder. In this way the slightest hindrance to the passage of the strip can be detected and measured and its position recorded. The difference between the resistance encountered in the first and in the immediate subsequent introductions of the strip is taken as the measurement of physical attachment or adhesion of the tissues to the tooth surface. Preliminary measurements indicating resistances up to the equivalent of 30 gm. tangential dislodging force are encountered with a 1.0×0.1-mm. strip.

181. MORPHOLOGY OF INTERDENTAL GINGIVAL TISSUES.—*Jacques Kohl and Helmut A. Zander, Eastman Dental Dispensary, Rochester, New York.* The morphology of the gingival tissue located in interdental areas of monkey and human specimens was determined by buccal-lingual serial sections through this region. Drawings made of the outline of the interdental gingivae at $15\times$ magnification on Plexiglass sheets were assembled on racks, viewed, and photographed. Such a three-dimensional view of the interdental soft tissue shows a buccal and a lingual peak (papillae) connected by a "col" which has mesial and distal slopes. The col shape varies in the different interdental areas. These findings confirm Cohen's work on the subject. If the interdental gingival tissue is removed on one side of the jaw, within 2 months it will take on the same shape as the unoperated or control side. The epithelium covering the col area is

thin, non-keratinized, and shows a great deal of inflammation beneath it, in contrast to that covering the peaks.

182. Epithelial and Connective Tissue Reattachment Following Gingival Injury in Rats.—*S. S. Stahl and P. Person, New York University College of Dentistry, New York, and Veterans Administration Hospital, Brooklyn, New York.* Healing of a gingival wound requires reattachment of epithelium and connective tissue to tooth structure. This report presents a simple reproducible technique for study of these problems in small animals, which has permitted, to our knowledge, observation of hitherto undescribed cytomorphologic changes occurring during reattachment. Forty Long-Evans male rats of 293 gm. average body weight were subjected to wounding. Using a No. 6 round bur, a cavity was drilled into the mesial wall of the maxillary left first molar at the gingival margin, producing concomitant gingival and tooth injury. Animals were then sacrificed at various time intervals up to 30 days, and the healing process was studied histologically. It was noted that apical epithelial proliferation along the dentinal wall began approximately 7–9 days postinjury. Of particular interest in certain specimens was the occurrence of round epithelial cells (containing large, round nuclei) whose periphery was in direct adherence to the dentinal wall. In other instances, more flattened epithelial cells were seen adhering to the dentin by means of a cuticle-like structure. At the cementum, the epithelial cells appeared adapted to irregularities of the cemental wall and thus achieved epithelial-cemental adherence. Connective tissue reattachment to tooth structure occurred in a few specimens and was associated with lesser local inflammation. These observations suggest that, in the experiments described, initial epithelial attachment to dentin occurred by means of direct cellular adherence to the tooth, followed by flattening of the cells and cuticle formation.

183. Tonus of the Marginal Gingiva.—*Howard D. Beardmore, University of Alabama School of Dentistry, Birmingham.* In periodontal disease local defense mechanisms of the region of the gingival sulcus are as important as local irritants. The "tonus" of the free gingival margin against the tooth surface is one of the important defense mechanisms, in that it provides a mechanical barrier to food particles and bacteria entering the area; but, should any be forced into the sulcus, they are rapidly removed by the continuous minute flow of fluid, described by Krasse, from the depth of the crevice. This flow of fluid is rendered functionless without close apposition of tissues against the tooth surface. Interest is then directed toward those factors responsible for the maintenance of a high degree of tonus in the free gingival margin. A method of measuring tonus (the ability of the gingiva to withstand forces which would tend to dislodge it from the tooth surface) was therefore devised. A periodontal probe tip was mounted on a heavy spring inserted into a handle. Deflection of the spring by manual retraction of the gingival rim was measured by an extensometer micrometer (rectilinear transducer) calibrated in grams. Pocket depth was found to be inversely related to tonus, the degeneration of tissue concomitant with pocket formation reducing the mechanical strength of the investing gingivae. Pocket depth and gingival inflammation are not correlated, but it was found that tonus was inversely related to the degree of inflammation, which confirms Arnim's histologic findings and suggests an early serious and increasing breakdown in local defense mechanisms.

184. The Relationship between Occlusion and Periodontal Status.—*Donald R. Poulton and Sanford A. Aaronson, Department of Orthodontics, Western Reserve University, Cleveland, and Division of Orthodontics, University of California School of Dentistry, San Francisco.* The existence of a significant relationship between occlusion and periodontal status is of great clinical importance, but studies in this area have not been forthcoming because of the lack of a workable index of occlusion. A new occlusion index (the OFI) based on incisor overbite, posterior interdigitation, anterior crowding, and overjet measurements was developed by Poulton at

the National Institute of Dental Research and showed good agreement of scores by two examiners and a strong correlation with a separate need-for-orthodontic-treatment rating. The occlusion index (OFI) and a periodontal index (Russell) were applied to 908 young males, and the results analyzed statistically. When the two scores were plotted against each other, each category of increasing malocclusion score received a progressively higher average periodontal score. The coefficient of correlation formula was then applied to the data and r was found to be $+ 0.24$, which indicates a small, but significant, degree of correlation in a sample of 908. Each of the occlusion features was also compared separately with the periodontal score, and each showed a significant relationship. The relatively young age of the sample resulted in a very low average periodontal score (0.48), and it will be of interest to see in further studies whether the same relationship to occlusion exists in cases with more periodontal disease. Although many factors must be taken into account, the observed correlation between occlusion and periodontal scores indicates that malocclusion is an important predisposing factor in the etiology of periodontal disease.

185. Disuse Atrophy of the Periodontium in Molar Teeth of Mice.—*Sidney A. Cohn, Division of Anatomy, Medical Units, University of Tennessee, Memphis.* Right upper molars of 194 albino mice were extracted at 25 days of age, to observe the long-term effect of early loss of function on teeth without antagonists. Four experimental animals and three intact controls were sacrificed every 50 days thereafter. Molars were studied grossly, as well as microscopically. Macroscopic observations showed marked gingival recession and root exposure on teeth without function. Little cuspal wear occurred on these molars, and there was an intense blackening and staining of the enamel. Histologic sections revealed that loss of function alone, without any apparent infection, caused a remarkable and progressive atrophy of the periodontium, leading to its almost total disappearance by 800 days. Alveolar bone was extensively resorbed, particularly interdental bone. There was an apical proliferation of the epithelial attachment, a disorientation and loss of fibers of the periodontal ligament, and a considerable resorption of cementum. Only traces of periodontal tissues remained in the interseptal region at 800 days, and in several instances fusion of root tips of adjacent molars had taken place. Although supra-eruption was not apparent, there was a pronounced distal drift and narrowing of the interdental spaces. These severe atrophic changes are interpreted as being due to the almost complete lack of stimulation of the periodontal tissues by occlusal contact. The chronologic sequence and pattern of these degenerative changes in the tissues of the periodontium will be discussed and compared with similar, but less pronounced, changes when one or more opposing teeth are present.

186. Relation of Protein, Carbohydrate, and Fat Intake to Periodontal Syndrome.—*J. H. Shaw and D. Griffiths, Harvard School of Dental Medicine, Boston, Massachusetts.* Five experiments with a total of 324 rice rats were conducted to determine the influence of variations in the absolute amounts and in the ratios of protein, carbohydrate, and fat on the incidence of the periodontal syndrome. Complete or partial replacement of the sucrose in ration 700 by dextrin had no influence on the periodontal syndrome. Complete replacement of sucrose by ground rice resulted in highly significant reductions in the soft-tissue manifestations of the periodontal syndrome but much less striking reductions in calcified-tissue involvement. Isocaloric replacement of more than half of the sucrose in ration 700 by lard resulted in highly significant reductions in the incidence of the periodontal syndrome in the soft tissues. Almost complete prevention of soft-tissue lesions occurred when all sucrose was replaced by lard. Less striking reductions in the degree of calcified tissues resulted from sucrose replacement by lard. Replacement of casein by egg albumin at the 24 per cent level in diet 700 or the use of 12 per cent of either casein or egg albumin had no significant influence on the incidence of the periodontal syndrome. Various periods of feeding a carbohydrate-free diet were used to prevent the initiation of the periodontal syndrome. These regimens

were followed by the usual periodontal syndrome–producing ration 700. The incidence of periodontal disease among these groups of rice rats was dependent on the length of time that ration 700 was fed and independent of the age at which diet 700 was begun.

187. Relation of Mastication to the Periodontal Syndrome in Rice Rats.—*A. M. Auskaps and J. H. Shaw, Harvard School of Dental Medicine, Boston, Massachusetts.* Two experiments were conducted to determine the relation of masticatory function to the periodontal syndrome in rice rats. Three groups fed ration 700 comprised the first experiment: Group 1, controls; Group 2, left mandibular incisor amputated repeatedly to prevent occlusion with left maxillary incisor; and Group 3, both mandibular incisors amputated to prevent occlusion. A slight trend was observed toward an increase in the number of areas affected by the periodontal syndrome and in the rate of progression of the lesions in Group 2 and 3. However, these differences were not statistically significant, in view of a high variability within groups. The average weights of the sublingual and submaxillary glands of the rice rats in Group 3 were significantly increased over the weights of the same glands in the control rats (1 per cent level). Moderate weight increases in thse glands were noted for the rats in Group 2, but these differences were not statistically significant. The second experiment was composed of five groups of rice rats: Group 1, controls fed ration 700; Group 2, controls fed laboratory chow; Group 3, ration 700 with molars in upper right quadrant extracted; Group 4, laboratory chow with molars in upper right quadrant extracted; and Group 5, mineralized milk as the total diet. The average values for the number of areas and the rate of progression of the periodontal syndrome were significantly higher for the right molars with no opposing teeth than for the left molars with opposing teeth in Groups 2 and 4. However, the incidence of the periodontal syndrome for the left molars of experimental rats on either laboratory chow or diet 700 did not differ from those of control rats. The periodontal syndrome in the rats in Group 5 fed the mineralized-milk diet was not significantly different from those fed diet 700 but tended to be more severe than for rats fed laboratory chow.

188. Periodontal Pathology in a Strain of Diabetic Chinese Hamsters.—*Gerald Shklar and M. Michael Cohen, Department of Oral Pathology, Tufts University School of Dental Medicine, and George Yerganian, Children's Cancer Research Foundation, Harvard Medical School, Boston, Massachusetts.* Spontaneous hereditary diabetes mellitus in Chinese hamsters (*Cricetulus griseus*) has recently been described by Meier and Yerganian (*Proc. Soc. Exptl. Biol. and Med.*, **100**:810, 1959). For some time we have been searching for the appropriate experimental animal to study the effect of diabetes mellitus on the oral tissues. Twelve animals with spontaneous hereditary diabetes served as the basis for this study. Twelve normal animals were utilized as controls. The duration of the diabetes ranged from 29 days to 14 months. All diabetic animals received insulin-replacement therapy. The jaws of both experimental and control groups were decalcified by electrolytic methods and imbedded in paraffin. Histologic study of the experimental animals revealed generalized osteoporosis, periodontal pocket formation with resorption of alveolar crestal bone, calculus deposition, and marked inflammatory infiltration into the gingival tissues. The control animals did not present these periodontal alterations.

189. Effects of Experimental Thyrotoxicosis and Myxedema on Periodontium of Rabbits.—*Marvin M. Rosenberg, Henry M. Goldman, and Esther Garber, Department of Stomatology, Boston University School of Medicine, Boston, Massachusetts.* The purpose of this study was to determine the effects of thyroid dysfunction on the periodontium of adult New Zealand rabbits. Thyrotoxicosis was induced by administering daily subcutaneous injections of thyroxine at a dosage of 0.25 mg/kg body weight. Myxedema was produced by administering subcutaneous injections of 1 millicurie of iodine 131 bimonthly over a period of 4–6 months. The determination of

thyrotoxic and myxedematous states were based on protein-bound iodine studies, oxygen consumption, and pulse readings. Gross findings in the thyrotoxic group include loss of weight, nervousness, and a red, smooth tongue. In the myxedematous group the skin was firm, inelastic, and dry; the fur was coarse. There was an increase in weight, sluggishness, and the tongue was enlarged, broad, and smooth. Histologic findings in the thyrotoxic group revealed a relatively unaltered gingiva; a cellular, well-organized, slightly thickened peridontal ligament; and a bone picture of relative activity. The myxedematous group revealed changes in the gingivae, which included hyperparakeratosis with some regions of keratosis; longer and more numerous rete pegs; and intracellular edema. The underlying connective tissue demonstrated interstitial edema and areas of fragmenting fibers, with moderate disorganization of the collagen bundles. The peridontal ligament showed a diminished cellularity, areas of hydropic degeneration and fragmentation, and interstitial edema. The bone presents a picture of relative inactivity, with reduced periosteal and endosteal bone deposition. The most striking feature was one of osteoporosis. The marrow spaces were found to be enlarged and more cellular.

190. The Effect of Increased Functional Forces on the Periodontium of Splinted and Non-splinted Teeth.—*Irving Glickman, R. Sheldon, and Jerome Smulow, School of Dental Medcine, Tufts University, Boston, Massachusetts.* A histologic study was conducted in 6 rhesus monkeys to determine the effects of increased functional force on the tissues of the periodontium and whether uniting teeth in a fixed splint affected the response of the periodontium to increased functional force. The occlusion was altered by constructing full-gold crowns, so that only selected teeth were in contact. Splints were constructed of 4 gold crowns united in 1 unit. Experimental periods of from 10 to 132 days were used. Excessive functional force over a short term (10 and 21 days) resulted in increased vascularity of the periodontal membrane, compression and necrosis of the fibers, necrosis and resorption of bone, and resorption of cementum. The location of the destructive periodontal changes corresponded to the direction of the increased force. Comparable destructive changes were induced in the periodontium of all teeth contained within splints in which only one tooth was in contact with its functional antagonist at the outset of the experiment. Bifurcation and trifurcation areas showed the most severe changes. Complete repair of the periodontium was observed in long-term animals (92 and 132 days).

191. Diet and Periodontal Disease in Dogs.—*Morris Ruben, John McCoy, Philip Person, and D. Walter Cohen, Bureau of Biological Research, Rutgers University; Veterans Administration Hospital, Brooklyn, New York; University of Pennsylvania, School of Dentistry, Philadelphia.* It had been noted in a colony of dogs used in protein-depletion studies and maintained on semisynthetic diets and commercial dog foods of very soft consistency that heavy calculus deposits were formed around the teeth. Twenty-two dogs between the ages of 6 and 8 years were selected for a year's observation while maintained on either or both of the above diets. The teeth of all animals were scaled prior to the observation period. After approximately 6 months on the soft-consistency diets, staining and sub- and supra-gingival calculus were evident on the teeth of nearly all animals. As it accumulated, solid bridges of the calculus developed, which, in some animals, completely covered the tooth crowns. There was an associated periodontitis, which, in its severest form, was manifested by a foul breath, marked gingival recession, bone resorption, and, in instances, spontaneous shedding of teeth. Particularly noteworthy were frequent occurrences of periodontal abscesses and epuli. In certain animals selected for further treatment, scaling of the teeth and curettement of the gingivae produced dramatic ameliorative gingival responses, closely resembling changes seen in human gingiva following their treatment. Clinical, roentgenographic, and histopathologic features of the periodontal tissue reactions will be presented.

192. Proteolytic Enzymes Produced by Flora from the Periodontal Pocket.—*Irving B. Stern and Inez Mandl, University of Washington, School of Dentistry, Seattle, Washington; Columbia University, College of Physicians and Surgeons, Department of Obstetrics and Gynecology, New York.* Recently, several papers report the elaboration of an intracellular "collagenase" from mixed bacterial cultures obtained from the periodontal pocket. Consequently, this experiment sought to screen the bacterial flora of the periodontal pocket for their ability to produce proteolytic enzymes capable of breaking down the fibrous constituents of connective tissue. Thereafter, only extra-cellular "collagenase-like" enzymes from isolated bacterial cultures were considered. The enzymes present in the pure cultures were precipitated with ammonium sulfate, and these precipitated enzymes were tested against azocoll, orcein elastin, and chicken feather keratin. Thirty-six of 746 samples taken from periodontal pockets produced extra-cellular enzymes which could lyse azocoll. From these, forty-one pure cultures and forty-one enzyme precipitations were obtained. All precipitated enzymes could lyse azocoll, two could lyse orcein elastin, and none could lyse keratin.

XVI. FLUORIDE STUDIES I. MARCH 25, MORNING, SECTION A

193. Fluoride Concentration in Human Calcified Tissues.—*Leon Singer, W. D. Armstrong, and C. H. Carlson, University of Minnesota, Minneapolis.* The fluoride concentrations in the anterior protuberance of the mandible, the superior iliac spine, and the shaft of the femur have been determined in specimens from each of eleven cadavers and compared with the fluoride content in the enamel, dentin, and cementum of the lower incisor teeth of the same individuals. The following ratios of fluoride concentrations in the ash were found: dentin/mandible, 0.75 ± 0.156; dentin/femur, 0.78 ± 0.132; and dentin/iliac crest, 0.58 ± 0.171. The ratios of the cementum/mandible, cementum/femur, and cementum/iliac crest were 1.80 ± 0.473, 2.14 ± 0.851, and 137 ± 0.588, respectively. These data, which are to be extended, will permit the prediction of the fluoride content of the skeleton from that of the dentin or cementum.

194. The Influence of Fluorides during Tooth Development.—*Jasvant Parikh, School of Dentistry, Georgetown University, Washington, D.C.* These experiments were designed to study the calcification pattern in developing teeth of animals receiving toxic doses of sodium fluoride by using histologic and contact microradiographic techniques. A total of 6 litters of young newborn rats was used in this study. Beginning on the third day postnatal, animals from 4 litters were administered sodium fluoride daily by subcutaneous injections, in doses ranging from 3 to 45 μg. F (336 ppm). One of the control groups was given equivalent amount of distilled water, while the other did not receive any treatment. The animals were sacrificed after receiving 2–15 doses of sodium fluoride. The control animals were sacrificed at the corresponding intervals. Jaws containing developing teeth were fixed in alcoholic formalin solution. Prior to paraffin imbedding and preparing sections, one of the jaws was decalcified by Morse's technique. Although the other was not decalcified, it was not possible to make serial sections from undecalcified jaws of animals over 10 days of age. Some of the serial sections were strained with hematoxylin and eosin and Masson trichrome. The remaining sections were used for contact microradiography by using Philips CMR 1-5 X-ray machine. The observations suggest alteration in the calcification of developing teeth in animals receiving high doses of fluoride. The ameloblastic layer appeared to be disorganized and showed as radiolucent areas in the microradiographs. The enamel matrix was fragile, and its calcification was retarded, as compared with the controls. The radio-opacity of matured dentin in these animals was altered.

195. Long-Term Ingestion of Low Dosages of Fluoride in the Dog.—*R. E. Wuthier* and P. H. Phillips, University of Wisconsin, Madison.* The objective of this

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experiment was to provide comparative information between dogs and other species on the effects of long-term ingestion of low dosages of sodium fluoride (F) in drinking water. Data were sought on the effects of F on general health, reproduction, F accumulation in calcified tissues, and its effects on dental health and bone pathology. Twenty-three female beagle puppies were divided into 5 experimental groups and fed a low-F (*ca.* 0.5 ppm) cariogenic diet and distilled water supplemented with various low levels of F. Treatment lasted approximately 3.5 years. No adverse effects from any of the F treatments were detected from any of the measurements used as criteria in this experiment. The incidence of dental caries was very low in all dogs. Two of the 5 dogs in the control low-F group displayed defective enamel formation, with evidence of corrosion and pitting of the enamel surfaces. These defects were not similar to caries. F accumulated in the calcified tissues of all the dogs. The concentrations attained were proportional to the level of F in the water. The uptake of F in the femurs of dogs receiving 1.2 ppm F in water was 7.0 times more than the uptake of F from the control diet. Within each animal, the amount of F sequestered varied markedly among the different calcified tissues analyzed. Further, the reproducing females sequestered from 15 to 90 per cent more F (depending on the calcified tissue) than did the non-reproducing females.

196. Release of Fluoride and Stannous Ion from Toothbrush Bristle.—*William C. Foster and Benedict B. Kimmelman, Laboratory of Clinical Chemistry, Misericordia Hospital, and Dental Department, Albert Einstein Medical Center, Northern Division, Philadelphia, Pennsylvania.* Toothbrushes fabricated of nylon bristle containing 2 per cent and topical applicator brushes fabricated of nylon bristle containing 6 per cent by weight of SnF_2 were tested for quantitative release of F and presence of stannous ion when soaked and when wet-stroked on a taflon slab and on acrylic veneers in the mouth. For detection of F, a modification of the method of McClendon and Foster was employed. Distilled water and non-homogenized saliva, pre-analyzed for F content, were the media used. Strokes were made in series of 10 and of 100–150. Periods of soaking were 10 and 30 minutes and 24 hours. Five to 8 per cent of the 5-mg. F content of each of 3 toothbrushes tested was released in the total of the first 3 series of 100–150 strokes, and less thereafter. Two per cent was released in the first 3 series of 10 strokes of the topical applicator brush. Soaking of 2 applicator brushes released about 0.6 per cent F content in 10 minutes; 0.3 per cent in the next 30 minutes; and a total 3 per cent in the following 24 hours. Variations in quantities of F release per series did not relate to percentage content of SnF_2 in the nylon or to whether saliva or water was the medium. Ten days' exposure of brushes in air had no apparent effect on the following release of F on stroking. No F was released from any brush following 24 hours' soaking. Tests for presence of stannous ion were conducted by the method of Murray. Stannous ion was present in 9 of 13 soaking and stroking solutions tested and in all the first 3 stroke-series of the toothbrushes.

197. The Use of a Stannous Fluoride–containing Prophylaxis Paste.—*Charles W. Gish, Charles L. Howell, and Joseph C. Muhler, Indiana State Board of Health and Indiana University, Indianapolis.* In the search for better, more effective, and more applicable materials or procedures in the field of topical application and preventive dentistry, a stannous fluoride–containing prophylaxis paste was tested for its anticariogenic effect. Approximately 500 children received either a prophylaxis (without stannous fluoride) each 6 months and served as a control, or a prophylaxis each 6 months, using a stannous fluoride–containing prophylaxis paste (the prophylaxis paste contained 50 per cent stannous fluoride). At the end of both 6 months and 1 year, the experimental group showed a significant reduction in dental caries when compared with the control group. However, after the second round of treatment, the study was discontinued because of the undesirable side reactions, such as nausea, vomiting, severe blanching, and taste, of the 50 per cent stannous fluoride–containing prophylaxis paste. In a new study designed on the basis of further laboratory evidence,

approximately 500 subjects were divided into 3 groups and received either a prophylaxis (without stannous fluoride) and served as a control; a prophylaxis using a stannous fluoride–containing prophylaxis paste prepared by mixing 10 cc. of an 8 per cent stannous fluoride solution and 1–2 drops of orange flavoring to 10 gm. of a stannous fluoride–compatible abrasive; or a prophylaxis using the stannous fluoride–containing prophylaxis paste followed immediately by a single application of an 8 per cent stannous fluoride solution. At the end of 6 months, both the experimental groups showed a significant reduction in dental caries, with the combination treatment showing the greatest reduction.

198. Topical Stannous Fluoride for Preschool Children.—*R. Burgess, Mrs. Wm. Mondrow, G. Nikiforuk, and F. Compton, Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada.* Three hundred children between two and a half and four and a half years of age were used to determine whether topical application of 8 per cent stannous fluoride solution reduces decay in the deciduous dentition. The stannous fluoride application was given to 150 children; the other 150 children, a control group, received a distilled-water placebo. All subjects received clinical and bite-wing X-ray examination from the same dentist, who was unaware whether a child was assigned to the fluoride or to the control group. The X-rays were interpreted without knowledge of the child's treatment group. Caries experience data were confined to the deciduous cuspids and molars. The dental hygienist performed a thorough prophylaxis for each child and applied fresh stannous fluoride solution or placebo to each alternate child. The same procedure was repeated 1 and 2 years later. At the end of 2 years, 229 children were still available to the project: 121 children in the fluoride group and 108 children in the control group. The control children had an average DEF surface increment of 2.70 and 6.14 after 1 and 2 years of the study, respectively. The stannous fluoride children had an average DEF surface increment of 2.30 and 4.60 after 1 and 2 years of the study, respectively. Thus the DEF surface increment was 15 per cent lower after the first year and 25 per cent lower after the second year for the stannous fluoride group than for the control group.

199. Laboratory Studies concerning the Synergistic Effect of a Single Topical Stannous Fluoride Treatment and the Use of a Stannous Fluoride-containing Dentifrice.—*Victor H. Mercer, Indiana University Medical Center, Indianapolis.* Muhler, in a 3-year clinical study, has shown the synergistic anticariogenic effect of a single stannous fluoride topical solution, applied each 6 months, and the unsupervised daily use of a stannous fluoride–containing dentifrice. In order to learn further of the mechanism of action involved in this clinical study, in vitro determinations were designed to show the reducing effect on enamel solubility of a topical stannous fluoride solution plus a stannous fluoride–containing dentifrice, compared with the topical stannous fluoride plus a non-stannous fluoride–containing dentifrice. Brushing comparisons between the standard multituft soft-bristle brush and an applicator designed to provide greater brushing friction with the enamel were also studied. Results showed the non-stannous fluoride dentifrice to increase the enamel solubility proportional to the time element in brushing, following pretreatment with a stannous fluoride topical solution. Under identical conditions the use of the stannous fluoride dentifrice resulted in a much lower acid solubility. Using the criteria set forth in this study to evaluate clinical susceptibility to dental caries we came to the following conclusions: (1) the use of a non-stannous fluoride–containing dentifrice results in brushing away protection to acid decalcification afforded the teeth by topical stannous fluoride; (2) the stannous fluoride–containing dentifrice prolongs the decreased enamel solubility of the teeth afforded by the topical stannous fluoride; (3) the endeavors of brushing and the length of exposure to the dentifrice are important factors influencing acid solubility.

200. Studies on the Duration of a Single Application of 8 Per Cent Stannous Fluoride in Rats.—*Donald E. Derrow, George K. Stookey, and Joseph C.*

Muhler, Indiana University Medical Center, Indianapolis. At the present time there have been no published reports concerning the duration of the effectiveness of a single topical application of an 8 per cent solution of stannous fluoride, as indicated by a reduction in the acid solubility of enamel. In this experiment three series of studies were performed in weanling rats in which the reduction in acid solubility resulting from a topical application was studied as a function of time. In the first series it was found that 77 per cent of the reduction had disappeared at the end of 1 week. It was also shown that only a 4 per cent reduction occurred at the end of 1 week if the teeth were brushed daily with a stannous fluoride–containing dentifrice following the topical application. The second series studied the reduction in acid solubility as a function of 24-hour increments of time. Data obtained from this series indicated that the reduction in acid solubility dropped sharply between the second and third 24-hour intervals following the topical application. The third series involved the effect of various combinations of multiple brushings and topicals on the duration of the effect of the topical applications. The data obtained in this series confirm the earlier findings that brushing with a stannous fluoride dentifrice maintains the reduction in acid solubility resulting from a single topical application of stannous fluoride and indicate that multiple brushings and topicals and various combinations may alter this effect.

201. Further Studies on Fluoride Absorption in the Rat.—*Daniel B. Crane, George K. Stookey, and Joseph C. Muhler, Indiana University Medical Center, Indianapolis.* Earlier reports on studies on fluoride absorption from these laboratories were performed on anesthetized rats and dogs and suggested that the rate of fluoride absorption may have been affected by the anesthesia. In this series of studies on rats and guinea pigs the rate of absorption was evaluated by intubation of the fluoride and the removal of the entire intestinal tracts after a specific time interval. Fluoride analyses performed upon the intestinal tract indicated the amount of fluoride absorbed during the time period. Data obtained from these studies indicated that neither ethyl ether nor sodium nembutal anesthesia had an effect on fluoride absorption and that the rate of fluoride absorption did not differ from that earlier reported, i.e., 50–60 per cent after 1 hour and 70–80 per cent after 2 hours. In addition to these studies, experiments on the effect of a number of variables upon fluoride absorption were conducted. These studies indicated that a 24-hour starvation period prior to the fluoride intubation resulted in a 32 per cent increase in fluoride absorption during the first hour after fluoride administration. The effect of a number of inorganic ions was studied. Calcium and magnesium were found significantly to decrease fluoride absorption, while molybdenum increased fluoride absorption.

202. The Relationship between Fluoride Deposition and Metastatic Calcification in Various Soft Tissues in the Rat and Guinea Pig.—*George K. Stookey and Joseph C. Muhler, Indiana University Medical Center, Indianapolis.* A relationship between soft-tissue fluoride content and soft-tissue calcification has been suggested but never demonstrated. In these studies to be reported, rats were divided into two groups, one of which received a low-calcium, low-phosphorus stock diet and the other received a diet fortified with calcium, phosphorus, and calciferol and known to induce metastatic calcification. In addition, half the animals in each group received 1.0 mg. of fluoride as sodium fluoride daily by stomach tube. After 30 days, the animals were sacrificed, the soft tissues removed, and the fluoride, calcium, and phosphorus contents of each tissue determined. A similar study was performed with guinea pigs. The results indicated that, with increased soft-tissue calcium, there was a highly significant increase in fluoride deposition. By far the greatest degree of calcification and corresponding deposition of fluoride was observed in the kidney, by means of both analytical data and histologic studies. A similar, but less pronounced, effect was found in the heart and liver. These data also indicate that the presence of fluoride resulted in a 125 per cent increase in the calcium content in the kidneys of those rats receiving the calcification-inducing diet. In the liver and heart the fortified diet resulted in a 50

per cent increase in the concentration of calcium. These data suggest a direct correlation between the deposition of fluoride and the deposition of calicum in soft tissues under experimental conditions which induce metastatic calcification in rats and guinea pigs.

203. Combined Anticariogenic Effectiveness of Topical Stannous Fluoride and the Use of a Stannous Fluoride-containing Dentifrice.—*Joseph C. Muhler, Indiana University Medical Center, Indianapolis.* This report summarizes the 3-year data for clinical studies designed to compare the synergism between topical stannous fluoride applied each 6 months and that of a stannous fluoride–containing dentifrice (Crest). The clinical results showed no decrease in effectiveness of the clinical procedure where both anticariogenic agents were used. These data confirm the results obtained at the end of the first and second year, suggesting that the use of multiple principles of prevention is more effective in controlling both new dental caries and existing dental caries than the use of only one effective procedure. These data also confirm the laboratory studies, which have shown that the use of a stannous fluoride dentifrice will help maintain the tin content of the carious enamel, thus preventing the decreased effectiveness known to occur when the single application is used only once a year.

204. Studies concerning the Effectiveness of a Stannous Fluoride Prophylaxis Mixture.—*Mack Mericle, Indiana University Medical Center, Indianapolis.* Previous studies by the United States Air Force have suggested that the combination of stannous fluoride with silex placed in solution with a silicone–mineral oil solution has a markedly antisolubility effect on whole teeth. Because of the high concentration of stannous fluoride used in these studies, additional investigation was conducted in order to determine whether different combinations of this mixture could be found which would result in similar antisolubility effects but in which lower concentrations of stannous fluoride were used. Extracted non-carious teeth were prepared for solubility determination by methods previously described. Dental pumice and silex pumice with and without silicone and mineral oil added were evaluated for their antisolubility effectiveness with and without the addition of stannous fluoride or sodium fluoride. Similar studies were conducted in which the stannous fluoride was placed in solution and in which lower concentrations of stannous fluoride were used. Such studies suggest that silicone itself does not reduce the antisolubility of the enamel and that pumice has a minor effect. The combination of a 50 per cent stannous fluoride–50 per cent silex mixture placed in solution with silicone and mineral oil reduces enamel solubility by 80 per cent. Similar combinations with sodium fluoride reduce solubility by 20 per cent. If the stannous fluoride is placed in solution with water and added to the silex, much lower concentrations can be used without decreasing the antisolubility effectiveness of the combination.

XVII. MICROBIOLOGY II. MARCH 25, MORNING, SECTION B

205. Effect of Antibiotics on the Oral Flora.—*Stanley Handelman, Roland Hawes, and Rosario Yap, Eastman Dental Dispensary, Rochester, New York.* The purpose of this study was to determine the effects of long-term parenteral administration of antibiotics on the oral flora. The subjects in this study are grouped as follows: Group I, 92 children who had had rheumatic fever receive 250,000 units of penicillin daily by mouth. Group II, 65 siblings of Group I children as untreated controls. Group III, 35 children who received from 1,000,000 to 3,000,000 units of penicillin, and/or 1 gm. of tetracycline daily. Group IV, 21 children who had completed therapy, as in Group III, 1 year ago. Group V, 32 siblings of Groups III and IV subjects as untreated controls. Paraffin-stimulated saliva was collected and inoculated into differential media suitable for estimating the numbers of "total" aerobes, "total" anaerobes, streptococci, micrococci, lactobacili, veillonella, coliforms, and candida. The resistance

of organisms in saliva to two concentrations of penicillin and tetraycline on antibiotic test disks was determined. In general, the results do not reveal profound differences among the mean counts of organisms cultured. This is significant when it is considered that subjects have had no shift in bacterial flora in spite of continuous long-term antibiotic therapy. Test disks of penicillin and tetracycline were used to compare the relative antibiotic resistance of the organisms from the saliva. Subjects receiving antibiotics showed slightly narrower zones of inhibition when compared with the control groups. This was more marked in Group III patients receiving high doses of antibiotics daily. Organisms from Group IV subjects, who had discontinued antibiotic therapy for a year or longer, showed zone widths similar to those of the control group.

206. Isolation of Gas-producing Organisms from Root Canals.—*Louis I. Grossman, University of Pennsylvania, School of Dentistry, Philadelphia.* Gas-producing organisms have been empirically stated to be present in infected root canals and to be the cause of pain and swelling, etc., in the case of acute alveolar abscess. In a series of 100 positive cultures which were routinely obtained from root-canal samples, 7 varieties of gas-producing organisms were isolated in 11 cases. They were identified as follows: diphtheroid bacillus, *Serratia marcescens, Streptococcus viridans, Candida albicans, Pseudomona* species, and *Staphylococcus aureus.* This study is being continued with the object of determining the incidence of gas-producers in cases of acute alveolar abscess. Cultures from infected root canals were made in brain-heart infusion broth to which 0.1 per cent Bacto-agar had been added. Where growth was present, 0.1 ml. of the broth was transferred to triple sugar broth for growth of gas-producing organisms. Of 100 samples, 12 showed growth in triple sugar broth, and 0.1 ml. of the latter was transferred to blood–tryptose phosphate agar for identification. One of the twelve samples yielded no organisms for growth, the other eleven were identified as stated above.

207. Preliminary Report on the Study of *Endamoeba gingivalis.*—*Grace R. Baird, Frank J. Orland, and Owen Keller, Zoller Memorial Dental Clinic, University of Chicago.* An ecological study of micro-organisms of the oral cavity has been under way for some time at the Zoller Clinic. The protozoan *Endamoeba gingivalis* is frequently found in association with bacteria of the gingival crevice. One strain of this small amoeba, obtained from a dental patient at the Lying-In Hospital, has been propagated for 16 months and is still flourishing. Although strains were grown in a variety of media, they were most successfully maintained in a Locke–egg albumen-egg–slant medium with the addition of a standardized amount of rice starch. Several methods of cultivating this protozoan were studied in the course of obtaining stock cultures; some of the associated bacteria were eliminated by the use of antibiotics in repeated transfers. Morphologic and physiologic studies of living and stained amoebae were conducted by cinematographic means. Electron-microscopic studies were made of the finer internal amoebic structures. Germ-free rats have been inoculated with *E. gingivalis* culture and accompanying bacteria. Further observations are to be made regarding the dynamic aspects of this micro-organism.

208. A System for the Defined Study of Mixed Cultures.—*Richard B. Parker and Marshall L. Snyder, University of Oregon Dental School, Portland.* Studies of bacterial ecology, whether of the mucous membranes, tooth surfaces, soil, or sea, have been hampered by the lack of a satisfactory procedure simulating a natural environment defined sufficiently to permit necessary control and measurement of the system. To investigate interactions between two or more species, we have developed a continuous culture technique based on kinetically sound growth theory. It is an open system with chemostatically limited initial stages for each organism, feeding into a common non-limited continuous growth cell at a second stage. This permits comparison at any point of the growth curve of the numbers and growth products of organisms in pure culture with the numbers and metabolites resulting from interactions between

these organisms in mixed culture. Data are presented which show the comparative growth of *Streptococcus salivarius* and *Veillonella alcalescens* as pure and mixed cultures in both closed and open system.

209. A Study of 31 Strains of *Bacteroides melaninogenicus*.—*Sylvia J. Sawyer, Ronald J. Gibbons, and John B. Macdonald, Forsyth Dental Infirmary and Harvard School of Dental Medicine, Boston, Massachusetts.* *Bacteroides melaninogenicus* is a regular inhabitant of the oral cavity of man. It is a Gram-negative, strictly anaerobic coccobacillus which produces a black pigment from hemin. Thirty-one strains were studied in respect to their biochemical and metabolic characteristics. Fourteen strains were from the oral cavity and 14 from the intestinal tract of man, and 3 were from the rumen of cattle. All 31 were uniform in the following respects. All were preteolytic, attacking both gelatin and reconstituted guinea-pig collagen. All produced H_2S. None reduced nitrate, and none was hemolytic. All but 5 required, or were stimulated by, hemin. The strains behaved differently in the following respects: 19 strains (all the intestinal strains and 5 oral strains) were nonfermentative; 9 strains (including the 3 rumen strains) fermented glucose, lactose, and galactose; 3 (oral) strains fermented glucose, fructose, maltose, sucrose, raffinose, and starch; 16 strains required, or were stimulated by, menadione; all but 4 strains produced indol. Production of volatile acids was determined for 12 strains. Strains which were non-fermentative (5) produced lactic, acetic, propionic, and butyric acids. Fermentative strains produced mostly lactic and acetic aids.

210. Quantitative Determination of Bacteria in the Gingival Crevice of Man.—*Anne C. Dale, Edward Rosenthal, Louis S. Bortnick, Gun Axelsson, and John B. Macdonald, Forsyth Dental Infirmary and Harvard School of Dental Medicine, Boston, Massachusetts.* Samples of material from the gingival margin and crevice of individuals with periodontal disease were collected in steel capsules and weighed. The samples were diluted, sonicated, and plated on various media to obtain counts as follows. For an estimate of total viable counts, heart-infusion–blood agar was used. For counts of *Bacteroides melaninogenicus* blood agar with menadione was used. Streptococci were counted on sodium azide blood agar. Fusobacteria were counted on Omata's selective medium. Four plates were used at several tenfold dilutions. In addition, total microscopic counts and spirochete counts were determined by counting organisms against a known dilution of polystyrene particles. No significant differences were found between the counts per gram from normal gingivae and those from periodontal disease cases. Total microscopic counts averaged 1.6×10^{11}/gm. Total anaerobic viable counts averaged 3.6×10^{10}/gm. Total aerobic viable counts averaged 1.5×10^{10}/gm. Counts of anaerobic streptococci averaged 3.0×10^{9}/gm.; facultative streptococci, 2.5×10^{9}/gm.; *B. melaninogenicus*, 9.5×10^{8}/gm.; fusiforms, 4.3×10^{7}/gm. Spirochete counts (determined microscopically) averaged 8×10^{8}/gm.

211. Fermentation Patterns in Dental Plaque Micro-organisms.—*Robert E. Gillis, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey.* The formation of acid from carbohydrate by micro-organisms in the dental plaque is recognized as an important contributing factor in the etiology of dental caries. The fermentation patterns of the microbial flora of the plaque are, therefore, of considerable interest. As a preliminary step in examining this problem, organisms were isolated from dental plaques on the teeth of a small group of patients. The plaque material was obtained by carefully isolating the teeth to prevent salivary contamination. Organisms were separated from plaque material by conventional streaking on brain-heart infusion agar, blood agar, and lactobacillus selection medium and incubating at 37° C. under varying conditions of oxygen tension. Most of the organisms isolated under these conditions were facultative with regard to their oxygen requirement. Pure cultures of these organisms were screened grossly for evidence of acid formation by growth in an enriched broth containing glucose and phenol red. Non-proliferating cells of those organ-

isms found to be capable of acid production from glucose were examined through the use of classical manometric techniques, to elucidate the probable pathway of glucose dissimilation. In this manner, it was possible to demonstrate the existence of both homofermentative and heterofermentative patterns of glucose dissimilation in organisms obtained from the same plaque. On this basis, it seems reasonable to suggest that the net acid formation of a plaque is at least partially the resultant of participation of organisms utilizing these two patterns of glucose dissimilation.

212. Antibacterial Effect of Medicaments Incorporated in Pulp-capping Agents.—*George W. Burke, Jr., and Holmes T. Knighton, Medical College of Virginia, School of Dentistry, Richmond.* Antibacterial effects of certain antibiotics and germicides used to treat pulp exposures were studied. Dental pulps of first maxillary molars from 168 albino rats, weighing approximately 150 gm. and between the ages of 75 and 105 days, were exposed under aseptic conditions. Pulps from right molars were treated with either antibiotics, antiseptics, or a combination of both just prior to capping with zinc oxide–eugenol. Pulps from control left molars were capped with zinc oxide–eugenol only. Within 10 minutes, bacteremias were induced by intravenous injections of known quantities of *Staphylococcus aureus*. Earlier experiments denoted that these organisms were sensitive to the agents tested. One day later, pulpal tissues were obtained under aseptic conditions, incubated in broth suspensions, and examined later for the presence of *S. aureus*, which was detected in 129, or 85 per cent, of 152 control teeth. Pulps treated with camphorated phenol or zephiran were positive for the test organism in 83–90 per cent of instances and thus proved relatively ineffective when compared with controls. By comparison the incidence following use of local antibiotics was as follows: penicillin, 60 per cent; bacitracin, 32 per cent; combination of penicillin and camphorated phenol, 32 per cent. Further control measures demonstrated that any carry-over of medicament to broth cultures used in incubating pulps was not effective in inhibiting growth of the test organism. This study is accompanied by a statistical analysis.

213. Quantitative Changes in Salivary Lactobacillus Bactericidin Associated with Age.—*Lloyd B. Austin and Bernard J. Zeldow, University of Washington, School of Dentistry, Seattle.* A lactobacillus bactericidin found in both human mixed, parotid, and submaxillary salivas has been described (*J. D. Res.*, **38:**798, 1959). Relative changes in the levels of activity as a function of age were studied in this investigation. Mixed saliva was collected from humans ranging in age from birth to twelve years. The newborn group included both premature and full-term infants. The specimens were assayed by using the quantitative bactericidin test. The results indicate that this antilactobacillus system is lacking in the mixed saliva of premature infants and is not apparent in the majority of newborns during the first 4 days of life. With increase in age, there is an increase in the amount of the bactericidin present in the saliva.

214. Observations on a Bacteriolytic Agent in Caries-immune Saliva.—*Gordon E. Green, Henry Ford Hospital, Detroit, Michigan.* A lytic agent found in caries-immune saliva which is active against oral lactobacilli and streptococci has been reported. The resemblance of the agent to antibody globulin was noted. Recent studies have shown a greater amount of salt-precipitable globulin in caries-immune compared to susceptible saliva, which is confirmed by spectrophotometric analyses. The lytic agent can be absorbed from crude globulin by lactobacilli and subsequently eluted from the cells, most effectively at acid pH. Gel diffusion studies in plates and tubes, using crude globulins and rabbit antisera, showed few differences between immune and susceptible salivas. Fractionation of the lytic agent from immune saliva by the cold-alcohol precipitation method resulted in inactivation. Study of ammonium sulfate precipitations resulted in improved purification and definition of three salivary globulin components. In the purified fraction, activity of the lytic agent increased with concen-

tration. Skin-test reactions in actively and passively immunized animals, using purified globulin fractions from immune and susceptible salivas as antigens, indicated that the lytic agent may be immunologically peculiar to immune saliva.

215. Histochemistry of Intranuclear Inclusions in Salivary Gland Virus-infected Mouse Liver.—*E. Grandel and N. G. Grand, University of Illinois, College of Dentistry, Department of Oral Pathology, Chicago, Illinois.* When CFW mice are injected intraperitoneally with salivary gland virus (SGV)–infected salivary gland suspensions, intranuclear inclusion bodies are found in the liver cells. After an incubation period of 4–5 days, the mice were sacrificed, and their livers removed. Under the experimental conditions employed, the inclusions are found to occur consistently in hepatic parenchymal cells and to bring about a great increase in the size of the cells and their nuclei. Thick liver-tissue slices were prepared, and the tissue samples here were rapidly immersed in a Dewar flask containing either liquid nitrogen or a mixture of dry ice and ethanol. Tissue sections 6 μ thick were cut in a cryostatt at $-20°$ C., placed on a slide, rapidly thawed at room temperature and air dried, and immediately subjected to histochemical procedures. The intranuclear inclusions in the virus-infected hepatic cells were positive for acid phosphatase, succinic dehydrogenase, beta-glucouronidase, non-specific esterase, DNA, polysaccharides, and the sulfhydryl groups of proteins. Alkaline phosphatase, mucin, RNA, and neutral fat did not show histochemical evidence of their presence. Appropriate tissue slices were employed as controls.

216. An Anaerobic Oral Filamentous Micro-organism.—*Else Theilade* and Marion N. Gilmour, Eastman Dental Dispensary, Rochester, New York.* Studies were made of the physiological characteristics of 19 strains of a Gram-positive filamentous micro-organism isolated from the materia alba of 9 subjects. Cultural conditions for isolation and culture maintenance were also determined. The media employed were Douglas' blood agar and a modified brain-heart-infusion agar with yeast extract and serum. The predominant cell type was short filament, 1.0–1.2 $\mu \times$ 15–35 μ. Longer filaments, as well as rods and chains of rods, occurred frequently. All strains were strict anaerobes. All were indole-positive; none reduced nitrate, liquefied gelatin, hydrolyzed starch, produced catalase, or formed acetylmethylcarbinol. Carbon dioxide was formed from glucose. Acid was produced by all strains from xylose, fructose, glucose, galactose, mannose, O-methyl-glucoside, lactose, maltose, sucrose, and raffinose, but not by any strains from salicin, rhamnose, cellobiose, glycerol, dulcitol, mannitol, or sorbitol. This uniform groups of organisms appears to differ morphologically and physiologically from all previously reported forms of oral filamentous bacteria.

217. Resistance of L Cells to Infection with *Herpesvirus hominis*.—*Robert V. Blackmore, University of Rochester, Rochester, New York.* Mouse fibroblasts (L cell strain 929) cultured in monolayer are suitable hosts for *Herpesvirus hominis*. When such cultures (grown and maintained in Eagle's medium supplemented with 5 per cent bovine fetal serum) were exposed to a standard inoculum of *Herpesvirus* (strain H.F.), the cultures were destroyed within 6 days, concomitant with high virus yield into the nutrient medium. The response of L cell cultures following primary exposure to *Herpesvirus* was modified by controlling their nutritional state prior to, as well as following, such exposure. Under certain conditions of nutrition, relatively little infectious virus was released, and few of the cells of the cultures showed a cytopathic effect (CPE). When infectious virus could no longer be demonstrated (23 days after primary exposure), these cultures proved to be resistant to gross CPE when reinfected under the standard nutritional conditions in which control cells were destroyed. Such "induced" resistance to CPE and to viral multiplication was not maintained as a characteristic of these cells in subculture.

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XVIII. SALIVA AND SALIVARY GLANDS II. MARCH 25, MORNING, SECTION C

218. ACINAR DUCT TRANSPORT IN THE DOG'S SUBMAXILLARY SALIVARY GLAND.—*Basil L. Henriques, Tufts University School of Dental Medicine, Boston, Massachusetts.* The purpose of this study was to determine the relative contribution of the ducts and acini in producing the concentrations of Cl, HCO_3, PO_4, and Ca in the submaxillary saliva of the dog. This was achieved by comparing the net transport of each ion by acini and ducts separately. The method of stop-flow was effective in demonstrating the relative functions of duct and acini, while the time-course study, starting from t_0, verified the function of the duct. The retrograde injection of $HgCl_2$, by destroying the transductal transport of ions, allowed observation of relatively pure acinar secretion, and, by difference, the duct's function was implied. It was observed that chloride is secreted or filtered through the ducts at approximately plasma concentrations and resorbed by the duct cells. Next, bicarbonate is transported into the saliva in the acini, as well as in the ducts, though some resorption occurs in the ducts. Then inorganic phosphate is evidently secreted by the acini and resorbed in part by the ducts, while calcium was brought into the saliva above plasma concentrations through the acinar cells. Evidently calcium is stored in the cells of the acini and ducts. It is therefore stored and secreted by the gland in a manner similar to potassium.

219. A TIME-COURSE STUDY OF Na, K, AND FLOW RATE FOR THE PAROTID SECRETION IN MAN.—*A. Coykendahl and Basil L. Henriques, Tufts University School of Dental Medicine, Boston, Massachusetts.* The purpose of this experiment was to determine the relationships of sodium, potassium, and flow rate in the parotid secretion of man, immediately following the initiation and termination of secretion. This was accomplished by a time-course study of these factors. Five subjects were used five times each. The procedure was to collect six succesive samples of resting saliva, followed by 5 minutes of stimulated saliva, and ending with five samples of resting secretion. During stimulation, saliva was collected in time increments ranging from 15 seconds to $1\frac{1}{4}$ minutes, depending on the flow rate of the subject. This gave samples of saliva of no less than 0.2 ml. each. It was observed that the sodium concentration and flow rate responded rapidly to stimulation, running parallel courses in the three phases of secretion: resting, stimulated, and recovery. When the flow rate was low, so was the sodium concentration. When the flow rate was high, so was the sodium concentration. Conversely, the concentration of K during the period of stimulated secretion fell slowly in comparison with the rise of Na. The mean delay from the peak of sodium stimulation to the first of the low K concentrations was 1.62 minutes. Potassium recovery was also slow.

220. SALIVARY GLANDS AND SERUM CALCIUM.—*Stanley R. Saxe and Leo M. Sreebny, School of Dentistry, University of Washington, Seattle.* Samples of blood were collected from the marginal ear vein of 9 young adult male albino rabbits 34, 18, and 11 days prior to scheduled surgical extirpation of their major salivary glands. The animals were fasted for 15 hours prior to the operation; another sample of blood was collected, and the rabbits were then equally divided into 3 groups: (1) control (nonoperated), (2) desalivated, and (3) sham-operated. Additional samples of blood were collected at 3, 6, 24, and 72 hours after the time of operation. The serum was analyzed for calcium by means of the Eriochrome Blue SE–E.D.T.A. titration method. Fasting was shown to lower significantly the serum calcium level in rabbits. Surgical intervention, whether by extirpation of the major salivary glands or by sham operations, potentiated the effects observed after fasting. No significant differences could be found between the desalivated and the sham-operated animals; the removal of the salivary glands, therefore, had no influence on the serum calcium level. These findings fail to support the "salivary gland hormone theory" promulgated by Ogata and Ito.

221. Salivary Gland Ligates—Food, Water, and Weight.—*Vera H. Jeter and George W. Wade, College of Dentistry, Howard University, Washington, D.C.* This work was done as a part of other studies concerning the effect of salivary gland ligations in Wistar male rats 90 days of age. All animals were housed separately. Twenty rats with salivary glands ligated were divided into 2 groups of 10 each. One group received dry food, and the other group received food that was soaked in water. All food was weighed dry in 60-gm. weights each day. Twenty manipulated (manipulated animals were sham-operated) controls and 20 normal controls in groups of 10 received dry and wet chow. Water consumption was measured in cubic centimeters, and food consumption was measured in grams on a daily basis for each animal. Animals were weighed once a week. This experiment lasted for 30 days. None of the ligated animals were lost, and only 1 manipulated animal was lost. By the fourth week, ligated animals were eating almost twice as much food in both the dry and the wet categories as the animals in other groups. Ligated dry and wet groups and also manipulated dry-food groups averaged 10 cc. more water per day. The least weight gain was in the ligated group with wet food, and the most weight gain was in normal group with wet food. There was not much difference in other weights.

222. Specific Gravity of Parotid Fluid.—*James F. McAnear, Ira L. Shannon, and Gerald M. Isbell, School of Aviation Medicine, USAF Aerospace Medical Center, Brooks AFB, Texas.* Specific gravity as determined, using the BANCO gradient balance. In order to study very low flow specimens, only 1 drop of sample was necessary. Two organic base liquids were placed in the gradient column (150 graduations over approximately 10 inches), and the column was rotated some 300 times or until the diffusion of the two liquids was such as to provide a satisfactory spread between standards. One drop of immiscible standard solutions (1.000, 1.005, and 1.010) was allowed to fall into the column, and the level read after 60 seconds. A like time was allowed for each test specimen to settle. Under acid stimulation, 450 parotid fluid samples were collected, and the means for flow rate and specific gravity were 0.324 ml/min and 1.0033, respectively. A series of five samples collected from each of 69 subjects were 0.22, 0.24, 0.35, 0.40, and 0.60 ml/min. The means for specific gravity were 1.0027, 1.0030, 1.0033, 1.0038, and 1.0042, respectively. This positive correlation between the two variables was significant. The specific-gravity relation of parotid to submaxillo-sublingual fluid will be discussed.

223. Water Content of Oral Fluids.—*Gerald M. Isbell, Ira L. Shannon, and John R. Prigmore, School of Aviation Medicine, USAF Aerospace Medical Center, Brooks AFB, Texas.* Water content was determined by a modification of the method of Paaby. Samples were dried overnight at 70° C. under a vacuum of 1.5 inches of mercury. On 30 duplicates of fresh human sera, a mean of 91.25 per cent was found, and the S.D. of the method was 0.055 per cent. For 100 subjects, parotid fluid collected under the stimulus of sugared gum had a mean water content of 99.281 (S.D., 0.193) per cent. In this group, flow rate was negatively correlated with water content (between subjects $r = -0.304$). Three samples of parotid fluid were collected from each of 22 subjects at varying rates of flow. Flow rate means were 0.762, 0.410, and 0.281 ml/min, and corresponding water-content means were 99.350, 99.480, and 99.520 per cent, respectively. In the individual flow rate, water-content correlation coefficients ranged from −1.000 to −0.936. The over-all negative correlation was significant (P −0.01). Pilocarpine stimulation led to a mean of −0.861 (P −0.01) in other subjects. The water-content relation of parotid fluid to submaxillo-sublingual fluid was also studied and discussed.

224. The Identity of the Polysaccharide Produced by Oral Samples from Sucrose Solutions.—*Joel A. Dain and R. S. Manly, Thannhauser Laboratory, Boston Dispensary, and Tufts University School of Dental Medicine, Boston, Massachusetts.* Several batches of polysaccharide were prepared according to Manly and Dain

by treating salivary sediment with sucrose. The samples were purified by precipitation with 68 per cent alcohol. The purified materials lacked mobility according to unidimensional descending paper chromatography, and no oligosaccharide could be observed. When the polysaccharide was hydrolyzed with varying concentrations of HCl (0.01–2 N) and chromatographed in a number of solvent systems, fructose was the chief monosaccharide observed, but occasionally traces of glucose were found. The IR absorption spectra of the polysaccharide resembled that of fructose. The material did not diffuse through a cellophane membrane. These findings indicated resemblance to a levan, which was further substantiated by tests according to the Dische-cysteine-carbazole method; the dry polysaccharide had an extinction coefficient equal in value to essentially pure ketohexose.

225. TYROSINE AND TRYPTOPHAN CONTENT OF HUMAN PAROTID SALIVA BY ULTRAVIOLET SPECTROPHOTOMETRY.—*I. Zipkin and G. R. Hawkins, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Maryland.* Human parotid saliva has been shown in this laboratory to have a characteristic absorption spectrum in the ultraviolet. All determinations were made on the TCA precipitate to obviate the interference due to uric acid and possibly indoles. Twenty-one specimens of parotid saliva were collected by Lashley cups from 15 healthy individuals under paraffin-gum stimulation. The precipitate obtained by adding 1.0 ml. of 30 per cent TCA to 4.0 ml. of saliva was dissolved in 0.1 N NaOH, and the ultraviolet absorption spectrum determined. The concentration of tyrosine and tryptophan was calculated from the absorption paterns by differential spectrophotometry. Aliquots of the same solution were also analyzed for tyrosine by the Udenfriend and Cooper procedure and for tryptophan by a modification of the Steers-Sevag procedure. Excellent correlation was found between the values obtained chemically and spectrophotometrically on the TCA precipitate, i.e., the correlation coefficients were 0.94 and 0.98 for tyrosine and tryptophan, respectively. The equations of the regression lines were $Y = 1.02X - 0.31$ and $Y = 0.81X + 0.35$ for tyrosine and tryptophan, respectively, where X and Y are the concentrations of the amino acid by chemical and spectrophotometric procedures, respectively. The means $\pm$ S.E. of tyrosine and tryptophan in the TCA-precipitable protein were 5.4 ± 0.38 mg. and 3.0 ± 0.29 mg. per 100 ml. of saliva, respectively, by chemical determination. Whole parotid saliva contained 7.0 ± 0.42 mg. per cent and 3.5 ± 0.29 mg. per cent of tyrosine and tryptophan. It is apparent, therefore, that almost all the tryptophan resides in the TCA-precipitable protein.

226. SPECTROPHOTOMETRIC DETERMINATION OF TYROSINE, TRYPTOPHAN, PROTEIN, AND URIC ACID IN HUMAN PAROTID SALIVA.—*G. R. Hawkins and I. Zipkin, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda, Maryland.* Previous studies have demonstrated a high correlation between the chemical and spectrophoto-

Disease	No. of Cases	Tyrosine*		Tryptophan		Protein		Uric Acid (WPS)
		TCA (PPT)	[TCA (PPT)/ (WPS)]	TCA (PPT)	[TCA (PPT)/ (WPS)]	TCA (PPT)	[TCA (PPT)/ (WPS)]†	
Periodontal	23	4.4	80.4	2.6	96.0	61.5	41.0	2.6
Arthritis	19	4.2	71.8	2.6	90.1	72.7	40.0	1.3
Gout	21	3.5	69.5	2.4	91.5	60.8	44.8	3.3
Control	21	5.4	77.1	3.0	85.7	66.3	38.9	1.8

* All concentrations given in mg. per cent. WPS = whole parotid saliva.

† Ratio in per cent.

metric determination of tyrosine and tryptophan in the TCA-precipitable protein of human parotid saliva. Continuing studies demonstrate a similarly high correlation for the protein and uric acid analysis by the two procedures. In addition, these constituents have been determined both in the TCA-precipitable protein and in the whole parotid salivas of individuals with periodontal disease, rheumatoid arthritis, and gout. No significant variations from controls are apparent in the tyrosine, tryptophan, and protein content of the parotid salivas of disease cases. The uric acid content of the saliva approximated 56 per cent of the blood concentration. The concentrations of the various constituents by chemical and spectrophotometric methods in the TCA precipitate for all cases were tyrosine, 4.4, 4.5; tryptophan, 2.7, 2.5; and protein, 65.6, 68.4 mg. per cent, respectively. For uric acid in the normal whole parotid saliva, the values were 2.0 and 2.0 mg. per cent respectively.

227. Histochemical Characteristics of Salivary Corpuscles.—*Gordon H. Rovelstad, United States Naval Dental School, National Naval Medical Center, Bethesda, Maryland.* Salivary corpuscles (leukocytes) have been studied by means of staining techniques and histochemical methods in an effort to characterize these cells and their contribution to the oral environment. Protein, nucleoprotein, carbohydrate, and some lipid were demonstrated within the cell structures. According to ultraviolet microscopy and the Feulgen reaction, the nuclei demonstrated marked DNA activity, as did occasional masses within the cytoplasm. The cytoplasmic granules demonstrated a high protein content, according to both ultraviolet microscopy and sites of enzyme activity. Some carbohydrate, probably glycogen, was demonstrated in the cytoplasm by means of the PAS reaction. Occasional lipid deposits were indicated by the Sudan dyes. All these constituents were distributed in a manner similarly described for blood leukocytes.

228. Studies on the Inhibition of *L. casei* by Parotid Secretion.—*Alexander C. Kerr and I. Leon Dogon, Forsyth Dental Infirmary and Harvard School of Dental Medicine, Cambridge.* Sterile secretion collected from human parotid gland ducts inhibits the growth of *L. casei*. This effect has been demonstrated by the "well plate" and "viable count after direct contact" techniques. Inhibitory effects were assayed by measuring the increase in optical density of a standardized inoculum of *L. casei* ATCC 4646, to which the sample was added. The antibacterial effect was not reduced by millipore filtration, lyophilization, or adjustment of the pH of the secretion to pH 8.0 or 5.5. It survived storage at 4° C. for up to 400 days. Activity was reduced slightly by freezing and thawing and was abolished by heating to temperatures above 75° C. Acitvity fell off rapidly when the final concentration of secretion in the assay was reduced below 12–6 per cent and fell off gradually when it was increased above 50–100 per cent. After either extraction the activity was found to be wholly in the ether-insoluble fraction. Treatment with increasing amounts of trypsin gradually abolished the effect. Dialysis against 15 vol. of deionized water or 0.01 M and 0.05 M NaCl removed the effect, but it could be recovered completely when the residue and dialyzate were recombined. The dialyzable factor passed a special actylated cellulose membrane, suggesting that its M vol. was less than 1,500. Passage through Dowex 50W-X 8 (H^+ form) resin columns did not affect the dialyzable factor, but it was removed or destroyed by Dowex 21K (C^- form) resin. The only anions which were found to restore the inhibitory effect when added to the nondialyzable residue were thiocyanate and iodide.

229. The Effect of Inorganic Ions on the Electrophoretic Pattern of Whole Saliva.—*Clary J. Fischer, George H. Wyshak, and David Weisberger, Harvard School of Dental Medicine, Boston, Massachusetts.* Our study deals with the attempted separation of the proteins of whole human saliva by the use of paper electrophoresis. Results on concentrated whole saliva demonstrated a pattern of 5–6 protein components. During the course of our investigation it became apparent that inorganic

ions played an important part in the electrophoretic pattern of saliva. Addition of sodium chloride to saliva prior to electrophoresis produced decreased mobility of the protein constituents. High concentration of sodium chloride resulted in an electrophoretogram exhibiting only a compact band at the origin and one heavily stained, broad band moving toward the cathode. As the concentration of the NaCl was decreased, an increased anodal mobility was evident. Short-term dialysis of the NaCl-treated samples against water restored the pattern to that seen with original samples against water and restored the pattern to that seen with original saliva, i.e., 5–6 protein bands. It would appear that the ions affected an aggregation of proteins, which limited the individual mobilities. Upon complete removal of the inorganic ions by prolonged dialysis or electrodialysis, a further increase in mobility obtained, and a resolution of whole saliva into 11–13 individual fractions became evident.

230. HUMAN SALIVA: ITS ANTIGENIC COMPOSITION.—*Leon B. Leach, George H. Wyshak, and David Weisberger, Harvard School of Dental Medicine, Boston, Massachusetts.* In a study of the protein composition of saliva, it has been shown by agar diffusion techniques that paraffin-stimulated whole human saliva contains several serum proteins. By using Ouchterlony–agar diffusion plates, concentrated saliva produces a visible antigen-antibody reaction (AG-AB) with rabbit antihuman gamma globulin. Similarly, saliva produces an AG-AB with rabbit antihuman serum consisting of many zones of precipitation. In an attempt to identify the antigenic composition of concentrated normal human saliva, Cohn serum protein fractions I, II, III, IV, and V were simultaneously used in the Ouchterlong plates. Zones of identity indicated some definite similarity with the various fractions. Because of the large number of antigen-antibody zones of precipitation within the agar and the resultant lack of discrimination of these zones, immuno-electrophoretic studies were undertaken. The results obtained with rabbit antihuman serum indicate that there are at least 6 independent antigenic substances common to saliva and serum. The identity of these antigens is currently being investigated.

XIX. E. H. HATTON AWARD COMPETITION II. MARCH 25, MORNING, SECTION D

231. EPITHELIAL ACTIVITY IN THE EXFOLIATION OF THE DECIDUOUS TOOTH AND THE EMERGING PERMANENT TOOTH.—*Howard W. Johnson, School of Dentistry, University of Kansas City, Kansas.* The relationship of the sulcus epithelium of the deciduous tooth to the forming sulcus of its permanent successor has not been described. This study, made on the anterior teeth of two monkeys, describes the sequence of changes which occurs. The odontogenic epithelium of the deciduous sulcus proliferated to isolate the deciduous tooth. The odontogenic epithelium was then replaced with oral mucosa. The odontogenic epithelium degenerated. In eccentric eruption of the permanent tooth, many fragments of the deciduous tooth remained after exfoliation. The fragments were encapsulated by epithelium, which arose from the stratum intermedium of the permanent tooth. In no instance did the sulcus epithelium of the deciduous tooth participate in the formation of the permanent tooth sulcus.

232. IS "PAROTIN" A HORMONE OF THE SALIVARY GLANDS?—*Louis P. Gangarosa, Rochester University, Rochester, New York.* The endocrine relations of the salivary glands were reviewed by Volker (*J. D. Med.*, **13**:125, 1958) and by Shafer and Muhler (*Ann. N.Y. Acad. Sci.*, **85**:215, 1960). These authors made little or no mention of extensive works reviewed by Y. Ito (*ibid.*, pp. 228–310) with regard to a protein, which he purified from the salivary glands, which he called "Parotin" and claimed to be a hormone. Ito claims for this "hormone" the following actions: (1) promotion of growth of hard and mesenchymal tissues, (2) lowering of serum Ca by 15–30 per cent, (3) changes in rabbit leukocyte counts following a definite time course, (4) effective in treatment of pyorrhea and arthritis, and (5) many other actions. Although much of their evidence appeared to be inadequate, we chose to give an agent with such important putative biological actions a thorough test. In a series of experiments using their

"hormone" preparation and designed to verify their results under controlled conditions, we obtained the following results: (1) no changes in serum Ca of dogs, rats, and rabbits; (2) no definite pattern of response in the leukocyte counts of rabbits or rats; (3) no changes in the growth rate, BMR, or incisor growth in rats; (4) no changes in the organ weight or histology of adrenals, parathyroids, testes, or femurs of rats; and (5) the same amount of activity as albumin in a test of anti-inflammatory potency. Our findings are supported by the work of Krainz (*ibid.*, pp. 310–12) on serum Ca of rats, and by Honjo *et al.* (*Endo. Jap.*, Vol. **4**, No. 3, 1957) on rat femur histology. We have no evidence for "hormonal" activity in the samples of "Parotin" examined.

233. The Measurement of Salivary Gamma Globulin.—*Stitaya Sirisinha, University of Alabama School of Dentistry, Birmingham.* An immunochemical precipitin technique was capable of measuring microquantities of gamma globulin in whole saliva and salivary secretions. A specific pooled rabbit antiserum was prepared against commercial (Hyland) human gamma globulin tested to contain 90.5 per cent 7S and 9.5 per cent 11.6S components. Salivary gamma-globulin nitrogen was calculated from the specific precipitate nitrogen determined by the biuret method standardized against micro-Kjeldahl. The average total error of the method was +2.2 per cent. Saliva did not alter the formation of the specific serologic precipitate, nor did it react non-specifically with the antiserum. The correlation coefficients among duplicate results (0.980 for whole saliva and 0.988 for parotid secretion) were indicative of a uniform distribution of gamma globulin in the supernates of salivary fluids.

234. The Synergistic Effect of Molybdenum and Fluoride upon Caries in the Rat.—*Robin A. Roberts, Indiana University Medical Center, Indianapolis.* There have been reports suggesting that molybdenum in the drinking water decreases caries in humans. It has also been shown that molybdenum increases the absorption and skeletal retention of fluoride in the rat. This study was performed to study the effects of fluoride and molybdenum upon caries in the rat. One hundred and sixty weanling rats were divided by sex and weight into four groups. The first group served as the control and received distilled water. The second group received 25 ppm molybdenum as ammonium molybdate in the drinking water, while the third group received 25 ppm fluoride as sodium fluoride. The fourth received drinking water containing both 25 ppm molybdenum and 25 ppm fluoride. After 130 days the animals were sacrificed, their femurs removed for fluoride analyses, and their heads scored for caries. The control group had an average caries score of 9.6 lesions per animal. When molybdenum was placed in the drinking water, the average caries score was reduced by 18 per cent to a value of 7.9 lesions. The presence of fluoride in the drinking water resulted in an average value of 6.5 lesions, a 32 per cent reduction when compared with the control value. When both fluoride and molybdenum were present, the caries score was further reduced to 4.6 lesions, a 52 per cent reduction when compared with the control group. Fluoride analyses on the femurs confirm earlier reports that molybdenum increases the retention of fluoride. The results of this study suggest that the presence of molybdenum enhances the anticariogenic action of communal fluoride in the rat.

235. Studies on the Effect of Fluoride on Bone Resorption.—*James R. Hudson, Indiana University, Bloomington.* The known effect of fluoride on the apatite structure and some clinical observations have suggested that fluoride may have an effect on bone resorption. In this study, 144 weanling rats were divided into two groups, one group serving as a control, with the other group receiving 1.0 mg. fluoride as sodium fluoride for 130 days. At this time each major group was subdivided into six subgroups. All animals were placed in metabolism cages, and all the animals except the control subgroups received an agent known to result in bone resorption. The agents employed were dietary ammonium chloride, hypervitaminosis D, hypervitaminosis A plus hydrocortisone acetate, and ethylenediaminetetraacetic acid. Metabolism samples and blood samples were collected periodically over a 20-day period. At the termina-

tion of this metabolism period, all the animals were sacrificed. Some animals from each subgroup were used to obtain histologic slides to verify bone resorption. The remaining animals were ashed, and the carcasses and femurs analyzed for calcium, phosphorus, and fluoride. The metabolism and blood samples were similarly analyzed. The data obtained from this study indicate that the agents employed vary in their ability to induce bone resorption and in the length of the lag period. These data further suggest that the deposition of large amounts of fluoride in the skeleton of the rat may decrease the ease with which the skeleton may be artificially induced to undergo bone resorption.

236. The Effect of the Placental Transfer of Fluoride on Dental Caries in the Rat.—*John Osborne, Indiana University Medical Center, Indianapolis.* There are several reports in the literature indicating that, under certain conditions, placental transfer of fluoride does occur but no indications of an effect on caries. In this study, forty adult female rats were divided into four groups and mated. Three of the groups received 25 ppm fluoride as sodium fluoride in the drinking water ad libitum, while the fourth group served as the control and received distilled water. Two of the three experimental groups were taken off the 25-ppm fluoride drinking water at birth and at weaning, respectively. The third experimental group received the fluoride solution throughout the 150 days of the experiment. At this time, the animals were sacrificed, their femurs analyzed for fluoride, and their heads scored for caries. The fluoride analyses confirmed earlier findings that placental transfer of fluoride did occur in the rat. The caries scores indicated that those animals reeciving fluoride throughout the study had an average score of 5.2 lesions per animal, a 43 per cent reduction when compared with the average score of 9.2 obtained in the control group. Those animals receiving fluoride only during the period prior to weaning had an average caries score of 7.3, a 21 per cent reduction when compared with the control group. The animals which received fluoride only during the embryonic period from conception to birth had an average caries score of 9.2 lesions per animal. These data indicate that the placental transfer to fluoride under these experimental conditions had no effect on caries.

237. In Vitro Studies on Fluoride Absorption.—*Eugene Dellinger, Indiana University Medical Center, Indianapolis.* A number of investigations involving in vivo studies on fluoride absorption have been performed in this and in other laboratories. However, in this series of studies an in vitro technique was employed in an attempt to shed further light upon the mechanism of fluoride absorption. The technique involved the removal of an intestinal segment, immersion in a buffered media, and the measurement of the fluoride concentration inside and outside the segment as a function of time. Numerous attempts on various segments of the intestinal tract failed to show any ability to concentrate fluoride, thus indicating that an active process might not be involved in fluoride absorption. Experiments were then performed in which the rate of diffusion of fluoride and of the segment was studied. The results obtained from these experiments showed that the amount of fluoride passing from the segment into the media (1) was a function of the area of the intestinal segment, (2) could be altered by temperature changes, (3) was a function of the concentration of fluoride in media outside the segment, (4) was influenced by the presence of certain ions, especially calcium and magnesium, and (5) varied from segment to segment along the gastrointestinal tract, being much less, for example, when the stomach was used.

238. Inhibition of Enamel Demineralization by Repeated Treatments with Sodium and Stannous Fluoride Solutions.—*E. E. Fischer, Tufts University School of Dental Medicine, Boston, Massachusetts.* The purpose of this study was to learn whether frequency of treatment with low concentrations of sodium or stannous fluoride, such as might be found in dentifrice, would affect the demineralization rate of enamel sections. Six freshly extracted teeth were collected from each tooth six sections were prepared, and all sections mounted between glass slides, using the technique of Manly *et al.* (*J. D. Res.*, in press). The sections were placed in a 0.5 M lactate buffer, pH 5.0, with outer enamel exposed. The treatment solutions used were NaF (0.027

per cent), SnF_2 (0.05 per cent), and distilled water, applied for 3–5 minutes, either once or twice daily. Kodachrome transparencies of each section were made at 72-hour intervals through a polarizing microscope. The rate of decalcification was determined in microns from measurements of the decalcification front on the image of the projected slide. No difference in the rate of penetration was observed between sections treated once or twice daily with water. All the sections treated with fluoride solutions showed less decalcification. Sections treated once a day with stannous fluoride solutions had a rate identical with those treated twice a day with sodium fluoride solution. Sections treated twice a day with stannous fluoride solution experienced the least amount of decalcification, amounting to 58 per cent of control at 264 hours.

239. Deep Cervical Lymph Glands and Fluorides.—*J. L. Hayes and W. L. Griffin, College of Dentistry, Howard University, Washington, D.C.* Swiss mice in groups of 5 males and 5 females received 10 and 20 ppm of NaF and CaF_2 by way of their drinking water over a period of 45 days. In addition, similar groups received distilled and tap water for the same period. Fluid consumption was measured daily and recorded, and mice were weighed once each week. The results of this study indicate that significant alterations were apparent in the afferent lymphatics and the deep cervical lymph glands that are associated with the submaxillary and sublingual salivary glands in those mice that received distilled water and the fluoride compounds. With distilled water, trabecular elements were increased, and, in the medullary part of these glands, cords of cells became prominent, and many immature lymphocytic forms were seen. More frequently in these instances and occasionally in the other experimental categories, pale staining central areas of these lymph glands were observed. Distention of sinuses and spaces within these glands were found where the fluorides had been given, and these changes were more marked at 20 ppm and especially with NaF. Where CaF_2 had been administered, blood sinuses were larger, and the afferent and efferent blood vessels were likewise more prominent. In such cases the lymph glands appeared to have a greater cellular population and stained deeper with hematoxylin. As compared with the other parts of this investigation, there appeared to be more early forms of lymphocytes where CaF_2 had been given.

XX. FLUORIDE STUDIES II AND ENAMEL SOLUBILITY
MARCH 25, AFTERNOON, SECTION A

240. Fluoridation, Dental Health, and Socio-economic Level.—*C. R. Castaldi, W. Quigley, and W. Zackeral, Faculty of Dentistry, University of Alberta, Canada.* Dental health was evaluated for 537 children ranging in age from six through twelve years and from three socio-economic levels—high, middle, and low. Comparison was made with voting results in two successive fluoridation plebiscites. There were significantly fewer decayed and extracted teeth and more filled teeth in the high socio-economic group compared with the middle and low. Total DEF was, however, the same. There were fewer decayed and missing teeth and more filled permanent teeth in the high socio-economic group compared with the low. The total DMF was significantly lower for the high socio-economic group. The dental health of the middle group was more like the high socio-economic group than the low. In two successive fluoridation plebiscites the high socio-economic group favored fluoridation by large majorities, while the low socio-economic group voted against fluoridation. The voting results in the middle socio-economic group ranged between the high and the low.

241. The Binding of Fluoride by Connective Tissues.—*Milton B. Engel, Norman R. Joseph, and Daniel Laskin, University of Illinois, Colleges of Dentistry and Pharmacy, Chicago.* The binding of fluoride by connective tissues was studied in the rat and rabbit by an electrometric method previously described. Rib cartilage and dermis were exposed, and measurements were made to determine the density of negatively charged colloid. The charge density was determined over the pH range of 7.4–2.2,

thereby yielding an in vivo titration curve of the tissue colloids. Then these measurements were repeated after applying fluoride anion (NaF) at 10^{-3} M. Normally, at pH 7, the negative charge density of cartilage is 0.16–0.17 equivalents/kg H_2O, and that of dermis is about 0.050 equivalents/kg H_2O. After fluoride, these are lowered to approximately 0.090 and 0.041 equivalents/kg H_2O, respectively. At pH values below 3, in the region of the isoelectric point of these tissues, the negative charge densities of the connective tissues were elevated following application of fluoride. Connective tissues behave as amphoteric charged colloids which can react with other charged and polar molecules. In the case of fluoride, it is thought that this anion combines with heterogeneous connective tissue proteins through electrostatic interaction, through hydrogen bonds, or, after a time, by forming covalent bonds. The field of the heterogeneous protein is thereby altered, affecting the uptake of protons by carboxyl groups, as shown by the titration curves. This must modify, simultaneously, all other reactions of the protein. The high concentration of fluoride (relative to blood) found in tissues, including oral tissues, probably represents fluoride largely bound by cellular and extra-cellular proteins.

242. Intestinal Transport of Labeled Fluoride, Chloride, and Water.—*W. D. Armstrong, P. Venkateswarlu, and Leon Singer, University of Minnesota, Minneapolis.* Renal excretion and skeletal sequestration are mechanisms for maintaining homeostasis of body-fluid fluoride concentration, and a slow rate of absorption of fluoride from the intestine may also operate to prevent large excursions in plasma fluoride content. Isotonic solutions in tritium-labeled water containing either 0.2 or 1.0 mEq/liter of fluoride and chloride, each labeled, respectively, with F^{18} and Cl^{36}, were circulated through the entire length of the small intestine of anesthetized rats for 30 minutes, and aliquots of the solution were removed at frequent intervals for counting. The results showed that H_3^2O was transported from the intestine more rapidly than Cl^{36}, which in turn was transported much more rapidly than F^{18}. Equivalent concentrations of Fe^{++}, Fe^{+++}, Ca^{++}, and Mg^{++} did not interfere with F^{18} movement from the intestine, but 10 times equivalent concentrations of Mg^{++} and Fe^{+++} reduced the transport of F^{18} from gut to blood. The rate of F^{18} transport was not different between the upper, middle, and lower segments of the intestine. It was also found that up to 40 ppm of fluoride did not alter the rate of oxygen consumption by everted loops of intestine.

243. In Vitro Evaluation of Protection by F^- and Sn^{++} Following Topical Application to Human Teeth.—*L. W. Wachtel, U. S. Naval Dental School, National Naval Medical Center, Bethesda, Maryland.* The relative effectiveness of fluoride ion and stannous ion in protecting the surface of a tooth against lactic acid attach was measured in vitro by using unerupted third molars. The amount of phosphorus dissolved by 0.005 N lactic acid at pH 4.0 was determined on half the coronal surface of the tooth and compared with the untreated half. Subsequent treatment and testing of the etched control half of the tooth provided a further comparison of the normal treated surface versus an etched surface. The protective action of 2 per cent NaF, 0.01 N HF, 2.4 per cent $SnCl_2$, 0.2–8.0 per cent SnF_2, and combined treatments of NaF or HF and $SnCl_2$ was measured. These measurements indicate that the protective effective of SnF_2 is due to the separate actions of fluoride ion and stannous ion on the tooth surface. The effect is considerably greater on etched surfaces than on normal surfaces. During a 24-hour test period on etched surfaces NaF and HF exhibited about 20 per cent protection; $SnCl_2$, 35 per cent; F^- followed by Sn^{++}, 65 per cent; and SnF_2 solutions, 85 per cent. A SnF_2-containing dentifrice did not show the same protection as an equivalent amount of SnF_2 in solution, the results suggesting predominantly a fluoride effect, with little or no stannous ion action. The protective action of all solutions on both sound and etched surfaces was diminished when treated surfaces were soaked in isotonic saline solution for varying periods prior to testing.

244. Seeding Effect of HA in Calcifying Solutions Containing Different Levels of Fluoride.—*F. Brudevold and A. C. Messer, Forsyth Dental Infirmary, Boston, Massachusetts.* The seeding effect of HA as studied by employing calcifying solutions containing different concentrations of calcium and phosphate at different pH levels. The formation of solid was assessed by measuring the decrease of Ca and P in the solution at different periods of time. The rate of seeding increased with increasing amounts of seeding material and with increase of Ca, P, or pH in the calcifying solution. Addition of as little as 0.2 ppm of F markedly increased the rate of seeding at pH 7.4, 6.5, and 6.2. The rate increased rapidly with increasing concentrations of F in the solution, and nearly maximal effects were attained before the solubility product of calcium fluoride was exceeded (8 ppm). Powdered enamel was less effective than HA in regard to seeding, but otherwise gave comparable results.

245. The Effect of Milk, Ascorbic Acid, and Calciferol on Fluoride Absorption and Metabolism in the Rat.—*David K. Hennon, George K. Stookey, and Joseph C. Muhler, Indiana University Medical Center, Indianapolis.* It has been reported on the basis of metabolic studies that the absorption of fluoride from milk is less than the absorption of fluoride from water. Since milk has been considered a vehicle for fluoride administration, it seemed worthwhile to compare the absorption and retention of fluoride when given in water or milk and when either ascorbic acid or calciferol was given simultaneously to weanling rats. Fluoride was administered by stomach tube as aqueous sodium fluoride and was given at the rate of 0.5 mg. per day. In all the groups receiving ascorbic acid or calciferol, the fluoride retention in the femurs and skeleton was comparable to that of the group receiving only fluoride and water. This indicates that neither ascorbic acid nor calciferol decreases the retention of fluoride. In addition, the group receiving fluoride, milk, and ascorbic acid showed the greatest fluoride retention of all those groups receiving fluoride. Metabolism data of feces and urine from each group revealed a close inverse correlation between the amount of fluoride retained and the amount excreted for each of the respective groups. A second series of studies was performed in which the absorption of each of the test solutions was directly measured by removal of the intestinal tract 4 hours after administrative and subsequent analysis for fluoride. These data indicate that in some instances the small increases in retention may be due to a direct effect upon fluoride absorption.

246. Alterations in Enamel Solubility and Calculus Deposition Produced by Coating Tooth Surfaces.—*Robert C. Caldwell, S. Wah Leung, Dan M. Guin, University of Albama School of Dentistry, Birmingham, and University of Pittsburgh School of Dentistry, Pittsburgh, Pennsylvania.* Intact enamel surfaces of extracted human teeth were coated with mixtures of Dow Corning 200 fluid (a polymerized dimethylpolysiloxane) and Eastman 910 adhesive (a cyanoacrylate monomer). An area of the surface was exposed to 0.20 M acetate buffer of pH 4.5 at 37° C. for 3.5 hours with stirring. Calcium dissolved from the enamel was determined by a versenate, Eriochrome black T method (T. Koulourides, M. S. thesis, University of Rochester, 1958). Resulting changes in enamel solubility in the table are expressed as percentage reduction in solubility as compared with uncoated enamel surfaces. The reductions

	Reduction (Per Cent)
Dow Corning 200 fluid	10 (negligible)
3 parts D.C. 200+ 1 part E. 910	38
1 part D.C. 200+1 part E. 910	63
1 part D.C. 200+3 parts E. 910	76
Eastman 910 adhesive	89

in enamel solubility were approximately 20 per cent less if the teeth were rolled together in water for 4 hours prior to determination of the enamel solubility. If the teeth were violently agitated (enough to cause enamel chipping), no reductions in solubility were observed as compared with uncoated teeth. The amount of artificial

calculus formation (S. W. Leung, *J. D. Res.*, **32**:689, 1953) on teeth coated with Dow Corning 200 fluid was the same as on uncoated teeth, whereas on teeth coated with Eastman 910 adhesive, artificial calculus formation was increased by 86 per cent.

247. A Study of the Pathways of Enamel Demineralization.—*E. Smith and M. B. Quigley, University of Alabama Medical Center, Birmingham.* Whether enamel decalcification occurs first between the rods or via the rods themselves is still controversial. It has been suggested that acid attack is via the interrod substance and chelation attack via the rod. This study was undertaken to compare natural "white spots" and artificially produced lesions, which have previously been shown markedly to resemble natural ones. These "white spots" were produced with sodium acetate buffer solution (pH 4.5) alone, buffer with gelatin, and buffer with agar. The tooth surfaces were abraded, and wax windows constructed so that both abraded and unabraded enamel surface could be demineralized. The unsectioned surface of the teeth was examine by dissecting microscope with reflected light directed at various angles to the surface and then coated with finger-printing ink. The teeth were then sectioned through the white spot, either parallel to or at right angles to the long axis of the enamel rods, and examined under the light microscope and with microradiography. The results showed that the surface seemed to be structurally intact, suggesting a molecular or ionic penetration of the surface by the demineralizing solutions used. Demineralization was much greater when the surface had been abraded. Microradiographs of rods in cross-section showed that in artificially produced "white spots" demineralization occurred in both rod and interrod, with greater demineralization down the rod.

248. Effects of Organic Anions on Decalcification Patterns of Enamel Surfaces.—*Geoffrey H. Sperber and Michael G. Buonocore, Eastman Dental Dispensary, Rochester, New York.* The effects of adding citric, tricarballylic, aspartic, malic, tartaric, and ethylene diamine tetraacetic acids in 0.01 M concentrations to 0.1 M acetic and lactic acid background buffers at pH 5.5 on the decalcification patterns of enamel surfaces in agitated and non-agitated systems was studied. Semiserial sections through the lesions were examined by microradiography and polarized light. In non-agitated buffers, radiolucent subsurface decalcification, with a radiopaque surface layer (resembling incipient caries) was produced on intact surfaces and, surprisingly, on enamel from which the outer surface had been removed (abraded enamel). In the background buffers this pattern was retained up until 2 weeks, with subsequent loss of the surface layer on continued immersion. The addition of tricarballyllic, aspartic, and malic acids allowed for the retention of the surface layer up to 4 weeks, suggesting that these acids are capable of retarding the dissolving action of the background buffers. The radiopaque surface layer produced on abraded surfaces was generally less resistant than the corresponding radiopaque layer on intact surfaces. After loss of the radiopaque surface layer, lesions consisted of a zone of enamel progressively less decalcified from the outer surface inward toward normal enamel. With citric and ethylene diamine tetraacetic acids, there was progressive surface loss, without accompanying subsurface decalcification. This type of lesion, resembling clinical erosion, was produced in agitated buffers with all acid combinations, irrespective of the patterns shown in non-agitated systems and for however short a period of exposure.

249. Effects of Some Food Factors on Enamel Solubility.—*Basil G. Bibby and Reginald J. Andlaw, Eastman Dental Dispensary, Rochester, New York.* Weight loss in blocks of bovine enamel in 6-hour fermentation mixtures of food and saliva has been used in conjunction with measurements of pH and titratable acid to indicate something of the nature of the factors in food retarding enamel decalcification. Additions of wheat germ or wheat bran to white flour produced increases in acid production and reductions in enamel solubility. The ash of germ and bran produced like effects.

Effects were also produced by water extracts of wheat germ. Milk powder or its ash increased acid production but reduced enamel solubility in salivary fermentations of sweet and semisweet chocolate. Except with bran, the pH curves of the food-saliva mixtures converged at 2 hours and fell to approximately pH 4.3.

250. Solubility of Neutron-bombarded Enamel in Dicalcium Phosphate–Flour-Saliva Mixtures.—*Donald J. Beck, Barrie R. D. Gillings, and Basil G. Bibby, Eastman Dental Dispensary, Rochester, New York.* To indicate whether caries reduction in animals, resulting from phosphate addition to the diet, is the result of modifications of enamel solubility or of changes in acid production, it was desirable to make continuous measurements of both factors in fermenting flour-saliva mixtures containing enamel and dicalcium phosphate. Conventional phosphorus or calcium analysis was unsuitable for this purpose, and, to obviate the difficulty, the radiophosphorus tag (P^{32}), produced by neutron bombardment of powdered enamel samples (120 mesh), was used to follow the conversion of the enamel in the fermenting flour-saliva mixture from the solid phase into solution. At various time intervals the amount of enamel dissolved was estimated from 100λ samples of a filtrate, using gas-flow proportional counting techniques. pH changes in the mixture were also recorded. The method proved to be both convenient and accurate and sensitive to small amounts (1 $\mu g/100\,\lambda$) of dissolved enamel phosphorus. The results of these studies indicated that the addition of dicalcium phosphate to a flour-saliva mixture produce a reduction in the solubility of enamel powder in vitro and that this effect was related in some degree to the amount of dicalcium phosphate added to the flour. However, the absolute differences in enamel solubility in mixtures ranging from 1 to 10 per cent dicalcium phosphate in flour were not pronounced.

251. Decalcification of NaF, SnF_2-treated Enamel Surfaces by Different Acids.—*Michael G. Buonocore and Geoffrey H. Sperber, Eastman Dental Dispensary, Rochester, New York.* Addition of 0.01 M citric and ethylene diamine tetraacetic (EDTA) acids to 0.1 M background acetate buffers at pH 4.5 and 5.5 influenced the decalcification patterns of treated and untreated enamel surfaces. Surfaces were separately treated with two 3-minute applications of a saturated NaF solution in pH 4.0 acetate buffer and with an 8 per cent SnF_2 solution. Both intact and abraded enamel surfaces were used. Semiserial sections through the lesions were studied by Grenz rays and polarized light. At pH 4.5 and 5.5, background buffers alone generally produced subsurface decalcification with a radiopaque surface layer on intact and abraded surfaces, with the latter more susceptible to decalcification. NaF application appeared not to retard decalcification, whereas SnF_2 did. At pH 5.5, addition of EDTA and citric acid to the background buffer accelerated decalcification of both intact and abraded surfaces, with production of cavitation. Again SnF_2 treatment appeared to increase resistance to decalcification. At pH 4.5, addition of EDTA resulted in complete surface loss with cavitation. In contrast, addition of citric acid at pH 4.5 resulted in retardation of surface decalcification, with preservation of intact surfaces but generally loss of abraded surfaces, accompanied by radiolucent subsurface decalcification. Decalcification retardation was greater with SnF_2 than with NaF treatment. Besides modification of enamel surfaces greatly influencing their decalcification patterns, the results indicate that not only does the decalcifying medium's composition determine rate and extent of decalcification but that the direction of these effects is a function of pH, as demonstrated by the apparent reversal tendency of citric acid's modifying effects between pH 4.5 and 5.5.

252. The Effect of Fluoride on Strontium Metabolism in the Rat.—*William E. Rogers, Edgar M. Wagner, and George Stookey, Indiana University Medical Center, Indianapolis.* The following experiment was performed to appraise potential benefits or hazards that might exist in the consumption of fluoride in an environment contaminated with bone-seeking strontium-90. The investigation was designed to

provide data both on the uptake and on the retention of calcium and strontium in rats at different levels of fluoride, under conditions which avoided the possibility of their interaction with fluoride in the gastrointestinal tract. Ninety-six weanling rats of matched weights were maintained in metabolism cages for 30 days, then half were sacrificed and the remainder were maintained for 30 more days and then sacrificed. During the entire period, the diet and fluoride was supplied separately by stomach tube. Two-thirds of the rats received a daily, intraperitoneal injection of a mixture of calcium-45 and strontium-89 during the first, or "uptake," period, but not in the second, or "retention," period. The remaining rats were water-injected controls. From each rat representative bones were removed, ashed, and analyzed for fluoride, calcium phosphate, strontium-89, and calcium-45. Metabolism samples were treated similarly. The results show that fluoride does not have a significant effect on the metabolism of strontium under the stated conditions.

XXI. GROWTH AND DEVELOPMENT. MARCH 25, AFTERNOON, SECTION B

253. PREVALENCE AND INTERRELATIONS OF MALOCCLUSION MANIFESTATIONS AND ETIOLOGIC FACTORS.—*R. M. Grainger, Division of Dental Research, Faculty of Dentistry, University of Toronto, Canada.* This is a preliminary report on the prevalence and interrelations of some 33 clinical manifestations and etiologic factors tabulated for 1,194 individuals in the control groups of the Burlington Orthodontic Research Center. The prevalence of the conditions is reported by age, anteroposterior dental relation, and anteroposterior skeletal relation. Some 800 intercorrelations are tabulated. The purpose of the study is to systematize knowledge of malocclusion in order (1) to check clinically derived knowledge, (2) to throw light on less understood malocclusion phenomena, and (3) to aid the assimilation of known facts by the students of orthodontics. The mathematical or epidemiologic approach provides a degree of objectivity to orthodontic research not achievable when drawing on memory of clinical experience, but becomes cumbersome when the number of factors being considered at one time rises above two or three. Two important concepts are demonstrated from the tabulations: (1) it is possible to detect mathematically the closely intercorrelated symptoms which represent clinical syndromes, and (2) the study of interactions demonstrates the concept of penetrance, i.e., the ability for specified untoward factors to produce a malocclusion only when other predisposing conditions are present.

254. MORPHOLOGIC TRAITS OF HUMAN MAXILLARY LATERAL INCISORS.—*Virginia M. Carbonell and A. E. W. Miles, London Hospital Medical College, Royal College of Surgeons, and Natural History Museum, London, England.* Certain morphologic characteristics have been observed in the dentitions of British skulls, 400 A.D.–1800 A.D. The material studied included Romano-British, Anglo-Saxon, and Early British skulls from later periods from various burial sites in England. The following observations have been made: (1) a high prevalence of shovel shape of the lingual surface of the maxillary lateral incisor, not always in association with central incisors of similar morphology; (2) a significant tendency for "boot-shaped" maxillary lateral incisors to occur in association with maxillary second molars of a particular type with a concave mesial coronal surface. Statistical treatment was applied to the data collected.

255. FUSED MOLARS AND SUPERNUMERARY MOLARS IN THE RICE RAT.—*D. Griffiths and J. H. Shaw, Harvard School of Dental Medicine, Boston, Massachusetts.* During 2 years in our rice-rat colony, 12 males and 12 females were found sporadically with fusion of the first and second molars in 1, 2, 3, or 4 quadrants. In 1 male, fusion of the 3 molars in 1 quadrant occurred. In 5 males and 4 females, a supernumerary molar was located distally to the third molar in 1 or more quadrants. From these rice rats a strain was developed with a high incidence of fused and super-

numerary molars. Among 236 descendants, 159 had fusion of the first and second molars in 1 or more quadrants, with no supernumerary molars; 1 male and 4 females exhibited a fusion of the 3 molars in 1 quadrant. In 45 offspring, supernumerary molars occurred in 1 or more quadrants; in 43 of these, fused molars were also present, while, in 2, supernumerary molars occurred without fused molars. In rice rats with at least 1 fusion, supernumeraries frequently occurred in quadrants with normal molars. The remaining 32 offspring had no phenotypic evidence of fused and supernumerary molars. Fused molars occurred most frequently in upper quadrants. No fusion in lower quadrants was seen when fusion was absent in upper quadrants. The degree of fusion varied. All external components of the first and second molars were readily identifiable in some fused molars, and their mesiodistal lengths were almost identical with the sums of the mesiodistal lengths of first and second molars. In others, considerable compression occurred, with reduction in size of the distal cusps of the first molar and mesial ones of the second molar, with over-all reductions in mesiodistal lengths. Histologic sections of representative fused molars demonstrated the presence of a single pulp chamber. Fusion of the first and second molars caused early eruption of the third molar of that quadrant.

256. Emergence of Permanent Teeth in the Monkey.—*V. O. Hurme and G. van Wagenen, School of Medicine, Yale Univerity, New Haven, Connecticut.* Completion of biometric analyses of long-term records on the permanent dentition of 73 normal macaques (*M. mulatta*), 31 males and 42 females, born and bred in captivity, makes this primate the first laboratory animal with emergence norms for both deciduous and succedaneous teeth. The ages used in computations are accurate within 0.04 year generally. Data on females were analyzed by conventional methods, but limited material on males necessitated a combination of indirect methods. Logarithmic probability paper was of great value in this work. Critical comparison of findings with those reported by other investigators led to the conclusion that most averages previously published are too high. Sex differences were noted in the emergence pattern of premolars and canines especially, and completion of dental arch formation was found to be tardier in the female than in the male, as a rule. Dentitions at one extreme of the developmental velocity scale were formed more than twice as rapidly as those at the other extreme. Tendency to variation was found to increase with age. Rate of odontiasis in the mulatta macaque is roughly triple that in man, but variability is less than a third. The data afford a hypothetical basis for equating life-expectancies in the two species of primates—23 years for the macaque, corresponding approximately to 70 years for man.

257. Experimental Induction of Cleft Palate with Cortisone in Inbred Mice.—*Robert J. Isaacson and A. P. Chaudhry, University of Minnesota, Minneapolis.* One hundred and four young virgin female mice of strain A were mated with littermates, and 68 females were isolated with vaginal plugs. These animals were then divided into six groups for respective treatments. The first three groups received 4 injections of cortisone in doses of 2.500, 1.250, and 0.625 mg., respectively; the fourth group received 2 injections of 0.15 cc. of 2 per cent formaldehyde; the fifth 4 injections of 0.15 cc. of saline; and the sixth group was left untreated. Injections were given at 6-hour intervals commencing at 11½ days of the gestational period. The animals were sacrificed at 17–18 days of gestation, and the teratogenic action of cortisone on embryonic secondary palate formation was clearly demonstrated. There was a cleft-palate incidence of 100, 85, and 32 per cent in the first three groups, respectively. One cleft palate was noted in the offspring of animals treated with formaldehyde. This suggested the possibility that an endogenous secretion by the maternal and/or fetal adrenal cortex in response to the stress of formaldehyde injection may have contributed to this teratogenic action. No isolated cleft palates were observed in the offspring of animals in either group 5 or group 6. However, an incidence of

3–10 per cent of spontaneous cleft lips in conjunction with cleft palates was observed in each group. This finding is in complete agreement with our previous experience.

258. SERIAL CEPHALOMETRIC STUDY OF THE GROWTH OF THE SOFT PALATE IN CLEFT-PALATE CHILDREN.—*Peter J. Coccaro, National Institute of Dental Research, National Institutes of Health, U.S. Public Health Service, Department of Health, Education and Welfare, Bethesda, Maryland.* Six hundred serial cephalometric roentgenograms, recorded from infancy through seven years of age, on 57 children with clefts of the lip and/or palate were obtained from the Cleft Palate Training Center of the University of Illinois. The technique employed in obtaining these roentgenograms has been described by Pruzansky and Lis (*Am. J. Orthodont.*, **44**:159, 1958). The growth in length of the soft palate was evaluated by measuring the linear distance from the posterior nasal spine of the hard palate to the tip of the uvula of the resting soft palate. A second measurement, employing the pterygomaxillary fissure landmark in place of the posterior nasal spine, was also evaluated for cleft-palate children. Comparisons were made with normative data on the growth of the soft palate as reported by Subtelny (*Plast. & Reconst. Surg.*, **19**:49, 1957). The following findings are noted: (1) Growth in velar length was noted from birth through seven years of age. It was marked and consistent until approximately 2½ years of age. (2) Periods of active and less active growth were strikingly similar for cleft palate and normal control groups. (3) Shorter velar lengths were observed at almost every age level for cleft-palate groups when compared with control groups. (4) When categorized according to cleft type, each group reflected similar growth patterns for velar length. The bilateral cleft-palate group tended consistently to show a slightly shorter velar length at most of the age levels studied when compared with the other two cleft-palate groups.

259. SOFT-TISSUE MORPHOLOGY IN THE CLEFT-LIP CHILD.—*John Mink and Charles J. Burstone, Indiana University, Indianapolis.* A comparison was made between normal and bilateral cleft-lip children in respect to (1) facial profile and (2) soft-tissue mass and posture of the lips. Tracings and measurements were obtained from oriented lateral head-plates in which visualization of both bone and soft tissue was aided by special diaphragms. Two basic lip positions were employed: (1) a relaxed position and (2) a position of light closure. The mandible was placed in centric occlusion. Significant differences were apparent in the integumental profile in areas comprising both upper and lower lips. The upper lip exhibited marked retrusion and lacked normal sulcus depth in the mid-line. The lower lip projected considerably anterior to its normal position. This protrusion was associated with the atypical lip posture concomitant with mandibular overclosure. There was no evidence that this protrusion was the result of hypertrophy in the lower lip. The greatest deviations in soft tissue were found in the upper lip from subnasale to labrale superius. Considerable variation, however, was present in this area. Some upper-lip thicknesses were greater and some smaller than normal. The normal sample did not exhibit such extreme variation. This variation in the cleft-lip child was related in part to the type, extent, and number of surgical procedures on the lip. The prognosis for achieving an acceptable aesthetic result by orthodontic treatment or a prosthesis in the cleft-lip child is strongly influenced by the soft-tissue morphology of the lips.

260. A STUDY OF VELOPHARYNGEAL CLOSURE USING CINERADIOGRAPHY WITH IMAGE INTENSIFICATION.—*Donald W. Warren, F. Allan Hofmann, and Richard M. Miller, Lancaster Cleft Palate Clinic, Lancaster, Pennsylvania, University of Pennsylvania School of Dentistry, Philadelphia.* Proper management of the cleft-palate patient is related to knowledge of the normal mechanism of velopharyngeal closure. Information was sought relating to possible relationships between (*a*) length of velum and pharyngeal depth; (*b*) length of velum and velar elevation during closure; (*c*)

pharyngeal depth and velar elevation during closure; (*d*) velar elevation differences during closure when phonating an isolated consonant, a vowel, and when blowing. Cineradiography with image intensification was used to record on film the velar and pharyngeal movements of 37 normal adult subjects during velopharyngeal closure. The films were analyzed and measured directly on a custom-designed tracing table, using the mid-sagittal plane for all measurements. The posterior pharyngeal wall was observed in all the normal subjects and compared with observations made on 385 cleft-palate subjects. A statistical analysis of the data from the normal subjects revealed that (*a*) there is no correlation between velar length and pharyngeal depth; (*b*) velar length is a significant determinant of velar elevation during closure (an inverse relationship exists); (*c*) pharyngeal depth appears unrelated to velar elevation (only during phonation of the vowel "oo" was a relationship found); (*d*) there is a significant difference in elevation of the soft palate for different sounds and for blowing. Film analysis of the normal subjects revealed no noticeable posterior pharyngeal wall activity during closure. Of the 385 cleft palate subjects, 122 (13.7 per cent) exhibited degrees of posterior pharyngeal wall activity.

261. Variations of the Facial Skeleton in Postadolescent Male and Females.—*Sidney L. Horowitz and Robert H. Thompson, Jr., School of Dental and Oral Surgery, Columbia University, New York.* Although sex differences in the adult facial skeleton are readily observed, most roentgenographic cephalometric investigations of facial morphology have been carried out on child or young adolescent subjects in whom quantitative sex variations in facial dimensions appear to be minimal. In this study, sex differences in the depth and height of the skeletal face were observed in a group of postadolescent subjects in whom facial growth was essentially complete. Data for depth and height of the face were obtained from cephalogram tracings of 100 males and 100 females. Criteria for selection of a subject in this study were (1) Caucasian, age twenty-one to thirty; (2) Class I (angle) dental occlusion with good alignment of teeth; (3) no prior orthodontic treatment; (4) excellence of the X-ray film. Measurements were taken of the sella-nasion-pogonion and gonial angles, as well as several linear dimensions; upper and lower face height; chin, mandibular, and sella-nasion lengths; and overbite. Males were larger than females in all dimensions studied except the gonial angle, in which the females averaged 124.8° and the males 121.8°, respectively. Differences between the mean values obtained for males and females were statistically significant at the 5 per cent level (*Z* normal curve test) in all the measurements but one—the overbite. Statistically significant differences (at the 5 per cent level) in the variability of measurements between the two groups occurred in only three of the nine dimensions studied—sella-nasion and mandibular lengths, and overbite (Fisher's *F* test).

262. Preliminary Observations on the Growth of the Condylar Cartilage Using H^3-Thymidine.—*Henry J. J. Blackwood, Royal Dental Hospital of London, School of Dental Surgery, Leicester Square, London, England.* When tritium-labeled thymidine is injected into animals, it is incorporated into the nuclei of those cells which are synthesizing DNA, and, by using tissue-section autoradiography, the sequence of proliferation and migration of these labeled cells may then be followed. This method was used to study the growth of the cartilage of the mandibular condyle of the rat and the kitten. Twelve August strain rats with an average weight of 60 gm. were each given a single intravenous injection of 25 microcuries H^3-thymidine, and 10 weanling kittens, with an average weight of 130 gm., were likewise given single intracardiac injections of 50 microcuries H^3-thymidine. The animals were sacrificed at 24-hour intervals from 2 hours to 8 days. Preliminary autoradiographic studies showed that from 2 to 48 hours the maximum number of labeled cells occurred in the center of the transitional zone of the cartilage but that, after 48 hours, the number of labeled cells in this zone decreased and at 96 hours labeled cells appeared throughout the hypertrophic layer. A somewhat similar pattern of cell labeling was observed

in the kitten, except that the kitten cartilage is penetrated by vascular canals. Labeled cells were found along the length of these canals, apparently independent of the general level of labeling of the cells within the cartilage.

263. Ectopic Eruption Pattern in Selected Permanent Teeth.—*William F. O'Meara, Department of Preventive Dentistry and Pedodontics, College of Dentistry, State University of Iowa, Iowa City.* Ectopic eruption of permanent teeth is regarded as a factor in premature loss of primary teeth. Review of available literature indicates absence of a detailed investigation of ectopic eruption as it affects more than one type of permanent tooth. The present study describes the pattern of ectopic eruption of central and lateral incisors and first molars. Three hundred and fifteen individuals with 771 ectopically erupting teeth of the above types fulfilled specified criteria. Group tendencies were as follows: (1) Nearly one-third of subjects experienced ectopic eruption of more than one type of tooth. The most frequent combination was of central and lateral incisors. Significant sex differences were noted. (2) Individuals having ectopic eruption of only one type of permanent tooth generally showed 2 or more teeth involved. (3) Relative frequence of eruption indicated that lower lateral incisors erupted ectopically most frequently, lower first molars the least. (4) Teeth tended to erupt ectopically as often bilaterally as unilaterally in the same arch, except for upper central incisors, in which bilateral involvement occurred twice as often as unilateral. (5) Quadrants in which ectopic eruption of a central incisor occurred revealed additional lateral or first molar involvement in about 20 per cent of upper and over 25 per cent of lower arch quadrants. (6) 20 per cent of subjects with ectopic eruption of an upper central incisor showed involvement of one or more lateral incisors or first molars in the same arch. Comparable analysis for the lower arch showed this tendency in almost one-third of the subjects.

264. The Parents' Reaction to the Birth and Early Care of Children with Cleft Palate.—*V. B. Tisza, B. Selverstone, and E. Gumpertz, Cleft Palate Institute, Tufts University School of Dental Medicine, Boston, Massachusetts.* Fifty-five unselected, consecutive cases of children born with cleft palate and/or cleft lip were studied by the child psychiatry group of the Cleft Palate Institute. In addition, the experiences and observations from a well-care clinic for babies born with these deformities were evaluated, and the reaction of mothers after giving birth to these babies was examined. Mothers react with a strong feeling of hurt and disappointment to the revelation that they have given birth to a congenitally damaged child. Cleft lip means more immediate trauma than cleft palate alone, because of the distressing appearance of the baby at birth. However, the significance of the hidden defect of cleft palate becomes obvious with the feeding difficulties, the irregular eruption of teeth, and later speech difficulties. Parents are also concerned about realistic difficulties, such as appearance, speech, and social acceptance. In addition, there are unrealistic fears rooted in the parents' individual problems and in popular prejudices; e.g., there is an almost universal apprehension about mental deficiency arising from the feeling that the head which is damaged from the outside is damaged from the inside as well. The parents who face such problems and challenge need help and understanding. Each case is thoroughly studied, discussed with the parent, and detailed recommendations are made as to the ways and methods of handling and supporting them through the child's growing years.

265. Synthesis Time and Generation Cycle of Oral Epithelium in Mice.—*Patrick D. Toto and Gokul Ojha, School of Dentistry, Loyola University, Chicago.* Thirty-six $C_{57} \times$ Black mice, 300 days old, were injected each with 60 μc. tritiated thymidine and sacrificed at intervals of 15, 30, 45, and 60 minutes and 2, 3, 4, 6, 7, 9, 10, 12, and 13 hours. The tongue was removed, cut sagittally, and fixed in formalin. Sections were cut at 5 μ, stained by the Feulgen method, and autoradiograms developed. Five slides in each period were selected, and the total mitotic figures and radio-

active-labeled mitotic figures were recorded. The percentage of labeled mitotic figures was calculated for each period of sacrifice and plotted on a curve. The 50 per cent points on the rise and fall of the curve were located at 3 hours and 11½ hours, respectively. The difference in time between these two points is 8½ hours. This represents the synthesis (DNA) time. The generation cycle was calculated at 96 hours.

XXII. PHYSIOLOGY. MARCH 25, AFTERNOON, SECTION C

266. PHYSIOLOGICAL AND PSYCHOLOGICAL CORRELATES OF A CHOLINESTERASE-LIKE SUBSTANCE IN PAROTID SALIVA.—*Donald B. Giddon and Vincent F. Lisanti, Harvard School of Dental Medicine, Brandeis University, Waltham, Massachusetts, and Institute of Stomatological Research, Brookline, Massachusetts.* Based on the observation of a reduction in a cholinesterase-like substance in the parotid saliva of hospitalized mental patients, a study was designed to ascertain the effects of systematically induced stress on this substance. Measurement of parotid cholinesterase, flow rate, blood pressure, and heart rate were obtained from ten psychologically evaluated college students for a period of 3 months prior to their being subjected to a replicated series of "stress" situations—i.e., blood sampling, hyperventilation, and cold pressor. Results indicated that the concentration of the salivary cholinesterase was depressed by anticipation of "stress," as well as by the actual stressors. The other physiological measures were more variable in their response and, in general, were not correlated with the cholinesterase-like substance or with each other under control or stress conditions. A tendency, however, for a pattern of physiological responses specific to each of the three stressors was noted. Significant correlations were also found between some of the physiological measures and the psychological data. Of special importance was the finding that only for the parotid cholinesterase was there a significant relationship between the change from the previously established control to the stress periods and a Rorschach measure of "emotional lability."

267. EXPERIMENTS IN TOOTH ERUPTION.—*Joseph M. Gowgiel, Zoller Dental Clinic and Department of Anatomy, University of Chicago.* This experiment is an extension of a serendipitous finding of a previous experiment in which it was observed that crowns or only partially formed crowns, resulting from external irradiation to the jaws of monkeys, erupted into the oral cavity. The experimental procedure was to administer a dose of 2,000 r single massive external irradiation to the jaws of 10-day-old Holtzman albino rats. Twenty experiments with 10 rats per experiment were carried out with the following results. Root formation was completely inhibited in the first and second molars, and the third molar crown was only partially formed; this produced rootless molars. Root formation of the lower incisor was temporarily arrested. The upper incisor was usually outside the field of irradiation and was therefore not usually involved; when it was in the field, however, root formation was also temporarily arrested. In spite of this cessation of root formation, all the teeth continued to erupt into the oral cavity. Histologically, it could be observed that the follicular sac was attached to the base of the rootless teeth and that the pulp chambers were closed before eruption was completed, with only a small amount of osteodentin formation. Osteodentin formation was a prominent feature in the lower incisors, where it was also found that odontoblasts preceded the ameloblasts in abnormal cell formation. The ameloblastic and odontoblastic cell layers involuted to a simpler form resembling squamous cells and mesenchymal cells and maintained this character while proliferating through an irradiation-produced vacuole on the periapical area. Then the epithelial cells began to proliferate and resemble ameloblasts, which induced the production odontoblasts, and a "second incisor" began to form.

268. LIVER OXYGEN CONSUMPTION OF DESALIVATED AND DUCT-LIGATED RATS.—*Ronald K. Bowman and David Bixler, Indiana University Medical Center, Indianap-*

olis. The study of tissue respiration by manometric techniques has been widely applied. Many aspects of salivary gland physiology and morphology have been studied and reported, including cellular respiration. However, little is known concerning the metabolism of other glands in the absence of salivary glands. For this reason, a study was undertaken to determine the effect of salivary gland removal and/or major duct ligation upon both endogenous oxygen consumption and aerobic glycolysis of liver homogenates. A standard Warburg respirometer was utilized in this study. Substrates were of two types: (1) Krebs-Ringer for endogenous oxygen consumption and (2) a fortified medium for aerobic glycolysis measurements. Experiment 1 consisted of three groups of animals (desalivated, duct-ligated, and control). Animals were sacrificed 30 days after surgery. Experiment 2 consisted of four groups—group 1 was desalivated and fed ad libitum; group 2 was desalivated and force-fed a highly nutritional liquid diet; group 3 was unoperated and force-fed the same diet, while group 4 was unoperated and fed ad libitum. The animals were sacrificed at 0, 20, 40, and 60 days after operation. The results indicate that either removal of the major salivary glands or duct ligation increases the endogenous oxygen consumption of liver homogenates. When the feeding conditions are rigorously controlled, no difference in endogenous oxygen consumption between operated and unoperated animals was noted. In the desalivated animals fed ad libitum there was an increased oxygen consumption at each experimental period. The significance of these results will be discussed.

269. Hormonal Influence on the Acidic Carbohydrates of Hamster Submaxillary Mucin.—*C. E. Klapper and J. M. Shackleford, University of Alabama Medical Center, Birmingham.* The acini of the submaxillary gland in the Syrian hamster are made up of only one type of cell, which cannot be classified by the usual standards as typically mucous or serous-secreting. Preparations stained by the common histologic methods do not reveal any sexual dimorphism. The amount and distribution of tissue carbohydrates are similar in these glands of both sexes, as indicated by the periodic acid–Schiff reaction. Alcian blue–stained preparations denote a much greater amount of acidic carbohydrates in the female submaxillary gland than in the male. Quantitative analysis shows a much greater total amount of sialic acid (an acidic carbohydrate) per gland, as well as more per milligram, in the female hamster than in the male. The total amount, as well as the concentration of sialic acid, is reduced to the normal male level in the submaxillary of ovariectomized animals. Conversely, a decided increase, close to the normal female level, was observed in males treated with estradiol. In all instances there was a close correlation between the intensity of the alcian blue–staining reaction and the amount of sialic acid. These observations indicate that the synthesis of the sialic acid fraction of the submaxillary secretion and/or its incorporation in the secretion is influenced by the female sex hormone. A sexual dimorphism can therefore be said to exist in the hamster submaxillary gland, at least in respect to the mucous fraction of the secretion.

270. Amylase Activity of Rat Submaxillary Gland Following Epinephrine Administration.—*Od Kridakorn, G. A. Schneyer, and L. H. Schneyer, Departments of Physiology and Dentistry, University of Alabama, Birmingham.* An increase in amylase activity in rat submaxillary gland, induced in vivo by parasympathomimetic agents has recently been described (*Ann. N. Y. Acad. Sci.*, **85**:189, 1959). The relationship of sympathomimetic agents, e.g., epinephrine, to gland amylase level has now been explored. Adult rats were given 10–50 μg. of epinephrine HCl subcutaneously at 30-minute intervals over a period of 3–3½ hours after removal of a control gland under light Nembutal anesthesia. Amylase activity in epinephrine-stimulated submaxillary gland rose to two- to threefold above that of the unstimulated gland (as compared with a ten to twenty fold increase induced by pilocarpine), while serum amylase levels remained unchanged. This effect is not reduced by dibenzylene (sympathomimetic blocking agent) but is inhibited by atropine (parasympathomimetic

blocking agent). It is concluded that, in rat submaxillary glands, the increase in amylase activity observed, following administration of a parasympathomimetic drug, is not mediated by release of a sympathomimetic agent, while effects of injected sympathomimetic agents are attributable to the appearance of a peripheral parasympathomimetic mediator.

271. Measurements of Metabolic Activity on Both Sides of Oral Microcosms (Dental Plaque).—*William A. Nolte and Sumter S. Arnim, University of Texas Dental Branch, Houston.* The purpose of this study is to measure pH and other changes on both sides of an organized, vital, oral microcosm (dental plaque). A plastic chamber, called "cariotron," maintains a microcosm of definite dimensions in a plastic washer between stainless-steel wire screens. Inlet and outlet tubes are located on each side of the washer. Foodstuffs, saliva, or other substances may be introduced or withdrawn from either side. Glass electrodes, stoppered in opposite sides of the "cariotron," fit close to the microbial mass. This allows a thin film of fluid between microcosm and electrodes. Metabolic end-products and other changes are measured by taking samples from the outlet tubes. Measurements of pH can be made simultaneously on both sides. Microcosms vary in their ability to form acids. Drops in pH may occur on one side more rapidly than on the other. When lemon juice is placed on one side, the pH on the opposite remains unchanged for about ½ hour, then it falls slowly. When powdered enamel is added on one side and glucose on the opposite, the pH drops on the glucose side but remains relatively unchanged on the enamel side. The microcosm acts as an individual biologic unit when vibrated into a unified gel-like mass within the plastic washer between the stainless-steel screens in the "cariotron." Characteristics of fresh individual microcosms are altered by environmental influences. Data obtained may reveal reactions that actually occur in microcosms within the human mouth.

272. The Effect of Parathyroidectomy on Saliva Calcium in the Rat.—*Leon Kraintz and Duane N. Andrews, University of Texas Dental Branch and Baylor University College of Medicine, Houston.* These studies were undertaken to determine whether salivary calcium levels reflect changes in serum calcium levels produced by parathyroidectomy in the rat. Male rats of the Holtzman strain were parathyroidectomized and maintained on a standard laboratory diet for 1 month. Whole saliva and blood samples were collected from pentobarbital-anesthetized control and parathyroidectomized animals. Pilocarpine nitrate was used to evoke salivation. Saliva samples were collected at 15-minute intervals over a 90-minute period. Microcalcium determinations were made on serum and saliva samples, using an adaptation of the EDTA method of Drehl and Ellingboe. While serum calcium levels of the parathyroidectomized animals were reduced to approximately 30 per cent below control level, saliva calcium levels were increased by approximately 30 per cent above the control saliva values. Control saliva calcium levels averaged 3.6 mg. per cent, while calcium levels of saliva from parathyroidectomized animals averaged 5.4 mg. per cent. It would appear that the calcium concentration of pilocarpine-evoked saliva from rats cannot be used as an index for blood calcium changes produced by parathyroidectomy.

273. The Metabolism of the Squamous Cells of the Gingiva.—*Robert E. Morgan, University of Alabama School of Dentistry, Birmingham.* In recent years several investigators have observed oxygen consumption in human gingivae as a means of studying the over-all metabolism of these tissues. Comparatively large samples of tissue had to be used, which were, of necessity, composed of all the cellular components found in the gingivae. It is important to study the metabolism of the individual tissues. The gingival squamous epithelium was chosen. Epithelium depends largely on diffusion of the nutritive elements through the basement membrane from the underlying connective tissue. Thus the rate-limiting factor in squamous-cell metabolism would logically be both qualitative and quantitative variations in the dif-

fused substances. Alterations in states of health of the gingivae should provide these differences. The technique chosen to permit the use of small numbers of dissected squamous cells was the Cartesian diver microrespirometer as developed by Lindstrom-Lang. This instrument is, in principle, a small flotation chamber containing the cells and substrate submerged in a variable-pressure fluid and gaseous mixture. Pressure changes in the flotation chamber due to oxygen consumption may be observed and indirectly measured by measurements of change in the over-all system. The effect of variations in the substrate media was studied on the metabolism of squamous cells from both clinically normal and diseased tissue by means of oxygen consumption. Experimental results on endogenous respiration and also with varying concentrations of glucose and alpha-keto-glutaric acid in the substrate indicate that the metabolic rate is largely independent of the concentration of metabolites in cells from clinically normal and diseased tissues.

274. Sulfate Metabolism in Rat Dentin.—*Gordon Nikiforuk and A. M. Hunt, Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada.* Twenty-nine 4-day-old rats were injected intraperitoneally with 0.5 μc of methionine S^{35} per gram body weight on each of 7 consecutive days. The animals were sacrificed when 17 days old. The first and second molars and incisors were removed, dried, pulverized, and the dentin purified. Radioactivity of all samples was measured in a stainless-steel planchet (7 cm^2), using a gas-flow system with a micromil window. Pure dentin, plated as an infinitely thick sample, had an activity of 3,400 counts per minute. The dentin powder was demineralized in a dialysis membrane against 0.1 N HCl for 2 days. Only a small fraction (1/20) of the original count was detected in the diffusate. The soluble organic fraction in the dialyzate was less than 1 per cent of the weight of the dentin powder and had an activity of 3,500 c.p.m., while the insoluble fraction represented over 15 per cent of the total weight and had an activity of 11,700 c.p.m. The absorption of the beta particles in the inorganic phase explains the relatively high activity of the demineralized organic fractions. The hydrolyzate of the soluble and insoluble organic fraction yielded active precipitates when barium chloride was added, indicating the presence of a compound (probably a chondroitin sulfate) containing a hydrolyzable sulfate group. The hydrolyzate of the insoluble organic fraction was chromatographed and the papergram scanned for activity. There appeared to be only one major activity peak, tentatively identified as the oxidized form of methionine.

275. Pulp Testing by Determination of Fusion Frequency.—*Dan Y. Burrill, Dental School, Northwestern University, Chicago.* In a search for clinical means of determining dental pulp condition, pulps were tested by applying square-wave and sine-wave impulses and determining the frequency at which the patient lost the sensation of separate impulses with the current just above threshold level. The square-wave impulses caused the threshold to change markedly; the sine-wave impulses were more satisfactory in this respect. Pairs of impulses and trains of higher-frequency groups of impulses were not useful. Determinations with the 0.001-second square-wave impulse gave a mean fusion frequency of 7.6 and median of 7.0 impulses per second for 200 clearly normal teeth, and a mean of 3.9 and a median of 3.5 for 25 teeth known to have pulp pathology. The longer sine-wave impulses gave a mean o 6.0 and a median of 6.5 for 145 normal pulps, and a mean of 4.34 and median of 3.9 for 92 pulps known to be pathologic. Some pathologic pulps gave a high fusion frequency, and some apparently normal pulps gave a low fusion frequency. A finding of a low fusion frequency in one tooth and a high fusion frequency in a comparable tooth in the same mouth appears, however, with fair reliability, to indicate pulp pathology in the tooth with low fusion frequency.

276. Ion Penetration through Teeth as Influenced by an Electrostatic Field.—*Ellery C. Stowell, James B. Taylor, and W. W. Wainwright, Veterans Administration Hospital, Long Beach, California.* The influence of electric current on the

movement of radioiodide and other ions from the surface of the tooth toward the interior was studied in radioautographs of tooth sections. A wire electrode from a battery was inserted into the enlarged root canal of an extracted tooth. The circuit was completed by placing the other electrode in a surrounding electrolyte solution. Potentials of the order of $1\frac{1}{2}$ volts were applied. In the case of I^{131} ions, when the negative pole is connected to the exterior of the tooth, complete penetration usually occurs in 1 hour. With the same experimental arrangement and no current applied, penetration is relatively slow, usually requiring 6 hours or longer to reach the pulp chamber. When the poles are reversed and a positive charge is applied to the surface of the tooth, no penetration is detectable.

277. Membrane Potentials of Pulpal Cells.—*D. C. Kroeger and F. Gonzales, University of Texas Dental Branch, Houston.* Little is known regarding the physiology of cells found in dentinal pulp. A cell which has been implicated in both the laying-down of calcium in dentin and the elicitation of pain is the odontoblast. Whether glandular or sensory, the membrane potential (E_m) should be greater than that of epithelium or fibroblasts. Using 3M KCl-filled ultramicroelectrodes, 10–40 megohms resistance, the membrane potential of many single cells was recorded from the exposed pulps of teeth of anesthetized dogs. The electrode was advanced through the pulp chamber with a micromanipulator. Conventional cathode-follower input, d.c. amplification, and oscilloscopic display were used. The potentials recorded indicate at least four groups of cells, two groups being similar to those reported for tissue-cultured pulps. As the electrode was advanced, a random recording of fibroblast cells was obtained with E_m of —8 to 12 mv. About midway in the chamber an occasional vascular smooth-muscle cell was impaled, E_m, —40 to 50 mv. External spike potentials of nerve fibers were occasionally obtained. As the electrode approached the distal wall, cells of the fourth group were regularly recorded, these cells having E_m of —35 to 45 mv, but showing no rhythmic depolarization. Histologic evidence indicates that these cells are the odontoblasts. Other studies include agents which cause depolarization of these cells and correlation with nerve impulses of pulpal afferents.

278. Sympathetic Nervous System Regulation of Salivary Gland Growth.—*Herbert Wells and Chester S. Handelman, Biological Research Laboratories, Harvard School of Dental Medicine, Cambridge.* Amputation of the lower incisor teeth of rats is followed by a marked increase in rate of growth of submandibular salivary glands. Previous work indicated this growth response to be closely related to the nervous enervation of the glands. Moreover, studies with dibenamine—an adrenergic blocking agent—indicated that the sympathetic nervous system might be involved not only in the response to incisor amputation but in the normal regulation of salivary gland growth as well. Utilizing surgical and pharmacological techniques, this possibility has been investigated further. For example, unilateral extirpation of the superior cervical ganglion, which deprives the gland of sympathetic nerves, slows the normal rate of growth and prevents the usual response to incisor amputation by the gland on the operated side. The contralateral gland is not affected. Unilateral cervical ganglionectomy also inhibits, ipsolaterally, the growth response of the glands which usually follows bilateral resection of the inferior alveolar nerves. Pharmacological studies with reserpine and bertylium toysolate also suggest an important role for the sympathetic system in salivary gland growth. Reserpine interferes with sympathetic function by depleting nervous stores of norepinephrine. Chronic treatment with reserpine, in low doses which do not affect body weight, blocked the response to incisor amputation and resulted in some atrophy of the glands. Bertylium toysolate, which interferes with release of norepinephrine by sympathetic nerves, also inhibits the response to amputation of the teeth. These studies support the idea that the sympathetic nervous system plays an important role in regulating the rate of growth of the salivary glands.

XXIII. SURGERY AND CLINICAL STUDIES. MARCH 25, AFTERNOON, SECTION D

279. REPAIR OF MUCA-GINGIVAL FLAPS IN THE RABBIT AND GUINEA PIG.—*Alfred Farchione, School of Denistry, Loyola University, Chicago.* Eight New Zealand white rabbits and 8 English smooth-hair guinea pigs were used this investigation. In the 7 experimental animals of each order the surgery was performed in the anterior labial region of the mandible. A supra-periosteal incision was made in the sulcus formed at the line of fusion between the free marginal gingiva and the oral epithelium. The flap which was created by this incision was approximately 4×4 mm. and was then sutured back to its original incision. The following sacrifice periods were used: 0 hour, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days. Histologic preparations were made by imbedding the specimens in celloidin and stained with hematoxlin and eosin. The following observations were made: (1) Bone apposition began as early as 2 days in the rabbit. At 3 days this bone clearly appeared as typical osteophytic bone. At 6 days the osteophytic bone formation stopped. In some areas the alveolar bone was increased in thickness by 2 or 3 times. (2) In the guinea pig the osteophytic type of bone formation did not begin until the fifth day. The thickness of the alveolar bone increased by less than one-quarter its original thickness. Using the same procedure it was found that in the order Rodentia the rate of healing and the amount of bone apposition was less than in the order Lagomorpha.

280. EXPERIMENTAL TRANSPLANTATION OF TEETH.—*R. Gordon Agnew, Chester Fong, W. S. Giles, and T. S. Grant, University of California School of Dentistry, San Francisco.* An intra-oral tooth transplantation project, of $3\frac{1}{2}$ years' duration, involved 50 operations (40 histologically processed) at 4 developmental stages, using rhesus or cynemolgus monkeys. Certain findings of the 31 autologous transplantations (unerupted mandibular third molar, mostly at stage of partial root formation, to first molar site) are presented. Routinely, no early reluctance to transplant acceptance was noted. Post-transfer retrograde pulpal phenomena did not progress to necrosis. Active periradicular and gingival repair paralleled pulpal cytologic and vascular reorganization. Non-tubular "osteodentin" of variable thickness lined pulpal walls, sometimes fully encompassing original root tip with roentgenographically visible calcified "hump." Moderate osteodentin pulpal lining was frequently overlain with tubular dentin (first irregularly, later regularly). Extending apically, this accomplished minor or substantial root elongation. However, root-tip coverage may occur without tubular dentin formation or substantial root elongation. Or limited root growth may accompany excessive intra-pulpal, even pulp-occluding, osteodentin deposition. Root extension may coexist with minor or major resorptions of original root structure, with, infrequently, perforations into pulp chamber or ankylosis with periradicular bone. Again, intra-pulpal osteodentin, without radicular elongation, may occur not only with pulpal cytologic and fibrillar changes but with bony trabecular formations, unconnected with pulpal walls, showing continuity with extra-radicular bone and at times extending into the pulp chamber. Similar phenomena may occur in transfers prior to root formation. Factors pertinent to the above phenomena would include operative sequelae; organizing influences of epithelium; mesenchymal activity; odontoblastic characteristics; stimuli relative to levels of calcified-tissue formation; vascular status; oxygenation, nutritional, and energy levels; etc.

281. HISTOLOGIC STUDY OF ALVEOLAR BONE REPAIR, UTILIZING TETRACYCLINE-INDUCED FLUORESCENCE.—*Philip J. Boyne, Georgetown University School of Dentistry, Washington, D.C.* Parenteral administration of tetracycline antibiotics is capable of producing heavily fluorescent incremental lines in newly deposited and calcified bone. In order to study the chronologic nature of osseous healing of extraction wounds under the altered environmental conditions imposed by implantation of bone-graft materials, tetracycline was given postoperatively to ten mongrel dogs. The drug was administered intravenously at intervals varying from 3 to 35 days after tooth removal

and alveolar implantation of homogeneous and heterogeneous bone-graft materials. Specimens were obtained at weekly intervals ranging from 3 to 12 weeks postoperatively. Ground sections were studied microscopically under ultraviolet light, and identical slides were examined microradiographically. Routinely stained specimens were also prepared. Each successive dose of tetracycline produced lineal yellow fluorescent patterns in the host bone, which were found to assume a characteristic arrangement in the area of the implant. Freeze-dried homograft implants demonstrated patterns easily distinguishable from those of the heterografts. Control sockets which were not implanted likewise exhibited characteristic fluorescence of new bone. Topical implantation of tetracyclines in the form of soluble dental cones produced a fluorescent compound in the initially deposited bone of the healing socket. Fluorescent patterns associated with bone implants were correlated with initial host acceptance, absorption characteristics, and rate of remodeling of the implanted bone.

282. The Replacement of the Mandibular Disk by Fibromuscular Homograft in Rabbits.—*Rudolph Sprinz, University of Sheffield, England.* The removal of the intra-articular disk in rabbits leads to a progressive condylar overgrowth associated with exostoses and depressions on the articular surface. It does not lead to regeneration of the disk. In an attempt to prevent postmeniscectomy changes, a fibromuscular graft (parotid fascia and maxillo-auricularis muscle) was inserted into mandibular joints after meniscectomy in rabbits. The graft covered the condyle completely and was secured with tantulum sutures. Sixteen animals were used. Macroscopic examination was made on 6, serial microscopic examination on 10. The experimental period was 8 weeks, the operations were performed unilaterally. Macroscopically, evidence of the graft was visible in 4 of the 6 joints. The condyles became larger than normal, exostoses and depressions were noted. Microscopically, a portion of the graft was observed in 9 of the 10 joints. The graft was mainly situated superiorly and adherent to condyle and/or squamosal bone. The graft survived in those parts of the joint where the bony surfaces were *not* close-fitting; when they became close-fitting, the graft merged with the perichondrium of the articular surfaces. On average, the remaining part of the graft was 3.9 mm. long and never completely compensated for the excised disk. The exostoses and depressions appeared more extensive than those following meniscectomy alone. The animals were weighed and radiographed weekly. Changes in weight and in intermaxillary distance indicated that the operations did not disturb the masticatory equilibrium.

283. Preliminary Investigation in Vestibular Fornix Extension Procedures.—*Harry M. Bohannan, University of Washington, School of Dentistry, Seattle.* This study was designed to provide an objective evaluation of the results achieved by three widely used operations for vestibular fornix extension. All past reports have been of a purely subjective nature, gained only from visual interpretation of postoperative clinical appearance. These reports, of conflicting nature, have resulted in some doubt as to whether any increase in depth can be achieved at all, and, if achieved, whether there is relative permanence of this alteration. The three basic operative techniques chosen for investigation were (*a*) complete denudation of the labial alveolar bone, (*b*) reflection of the mucosa but with periosteal coverage retained over the labial plate, and (*c*) vestibular incision into the base of the fornix. The procedures were carried out on thirty patients divided into three groups of ten patients each. Serial radiographic cephalometry, utilizing the Broadbent-Bolton cephalometer and a radiopaque lead shot positioned at the depth of the fornix, provided the method of evaluation. Records were made preoperatively and at regular intervals for a 6-month postoperative period. The movement of the lead shot as revealed by superimposition of the films indicated the postoperative alteration in vestibular depth as well as the pattern of healing. In all series an increase in vestibular depth was recorded. The mean gain in depth established for the denudation series was 5.1 mm., for the retained periosteum 1.0 mm., and for the vestibular incision 1.5 mm. The use of an acrylic stent did not affect the amount of extension attained.

284. THE USE OF POLYURETHANE IN FRACTURE FIXATION OF DOG MANDIBLES.—*Jaime Vrastorza, School of Dentistry, Georgetown University, Washington, D.C.* Chemical osteosynthesis has been applied to experimental fractures of the long bones with varying degrees of success. The purpose of this experiment was to test the effect of polyurethane osseous implants on openly reduced fractured dog mandibles. Nine animals were used. The mandibular posterior teeth on one side were removed in all animals, and the alveolar ridges were allowed to heal for 4 weeks. The mandibles of five dogs were fractured by Stryker saw on one side and a polyurethane splint placed across the fracture line. In three control dogs the mandibles were fractured in the same manner without placing a polyurethane implant. Animals were sacrificed at 3, 5, 8, and 10 weeks postoperatively. Resorption of the polyurethane was noted in varying degrees in all specimens. Bonding of the plastic material to host bone was minimal. Healing of fractures in some experimental animals occurred independently of the implanted plastic material. Postoperative infection with exposure of the implant occurred in two of the five experimental cases. The implant material appeared to possess sufficient strength and workability to effect fixation of the fractured fragments without actually contributing to the osteogenic process.

285. A STUDY OF THE RELATIONSHIP BETWEEN ORAL SEPSIS AND POSTSURGICAL COMPLICATIONS.—*Donald T. Waggener, Merle M. Musselman, Orval P. Nesselbush, and John O'Conner, College of Dentistry, University of Nebraska, Lincoln.* This study of patients prior to general surgery included physical examination, oral examination, and dental roentgenographic survey. Excluded from the study were patients with known active infections, non-dental foci, and those having obstetrical or gynecological surgery or incision biopsy. The group considered to have oral sepsis was deemed to have one or more of the following dental conditions: periapical infection, periodontal infection, pericoronitis, residual root infection, imbedded teeth in communication with the oral cavity, or an infected dental cyst. Postsurgical complications were grouped into one or more of the following categories: temperature elevation over 100°, wound infection, urinary infection, or respiratory infection. The patients were divided into two groups, one with oral sepsis (as defined for this study) and one without oral sepsis. Each group was subdivided according to type of anesthetic—general, local, or spinal. The groups receiving local or spinal anesthetics totaled 56 patients. Of the 80 per cent who exhibited oral sepsis, only 31 per cent had uneventful recoveries. The group receiving general anesthetics totaled 110. Of the 71 per cent who exhibited oral sepsis, only 15 per cent had uneventful recoveries, but, of the 29 per cent exhibiting no oral sepsis, 44 per cent had uneventful recoveries. The entire group totaled 166 patients, of whom 74 per cent exhibited oral sepsis, only 21 per cent of which had uneventful recoveries; 26 per cent showed no oral sepsis; and 51 per cent of this group had uneventful recoveries.

286. THE PRODUCTION OF MANDIBULAR FRACTURES: A STUDY IN HIGH-SPEED CINEMATOGRAPHY.—*Donald F. Huelke, University of Michigan Medical School, Ann Arbor.* Up to this time no one has seen a bone break, i.e., at a speed slow enough to enable one to determine the manner by which the specimen actually fractures. Previous investigations (Huelke, *J. D. Res.*, **38**:663, 1959; **39**:694, 1960) have determined the high tensile-strain areas (the areas which would fracture) in human mandibles, by using a non-destructive testing method (Stresscoat). To determine visually the validity of the Stresscoat results, high-speed moving pictures (2,000–3,000 frames per second) have been taken of fracture-producing impacts applied to the chin point of the mandible. In these studies, the condyles, as in the previous experiments, were firmly fixed and therefore unable to move, or were free to move in their base plates. Impacts varying between 6 and 12 foot-pounds were used. Frame-by-frame analysis of the film strips indicates that in all cases there are subcondylar fractures produced, along with fractures of the chin region in most cases. Angle fractures also are produced in those tests in which the mandible has both condyles firmly fixed in the base plates. Throughout all of these tests there was no evidence that the mental foramen is a structurally

weak area of the bone, as has so often been stated or implied in certain reports in the literature. These results dramatically and graphically illustrate and confirm the previous results in which the non-destructive testing method was employed.

287. Attempt To Produce Tooth Ankylosis.—*Philip L. Rubin and William Biederman, Murray and Leonie Guggenheim Foundation, Institute for Dental Research, College of Dentistry, New York University.* It is commonly believed that tooth ankylosis is caused by masticatory trauma. Several phenomena cast doubt upon this concept, e.g., the unemergent ankylosed tooth, relative rarity of opposing ankylosed teeth, and the absence of ankylosis in entertainers performing lifting and swinging acts with their teeth. The long way that tooth ankylosis has been effected experimentally thus far is by extraction and replantation. A similar result is the usual clinical consequence when a human tooth is avulsed accidentally and replanted. In this study, the left mandibular deciduous molars of 3- and 2-month-old puppies were subjected to injury of the paradentium as described below, and the analogous teeth on the right side acted as controls. (1) Lower left deciduous first and second molars. Openings were made, each about 3 mm. in circumference (No. 6 round bur), at the middle of the mesial root of the second and the distal root of the first, so that the cementum and interproximal area were slightly involved. (2) A repetition of No. 1. (3) Only the second deciduous molar was involved, but the area was greater and was cauterized with phenol. X-rays were taken pre- and postoperatively and again 3 or 4 months later, when shedding had been completed. The last series of X-rays showed no ankylosis of the experimental teeth, and there had been no significant variation in time of shedding as compared with the controls. It may be concluded that, although tooth ankylosis can be induced by extraction and implantation, direct trauma short of such radical procedure have failed. It would seem that the evidence does not substantiate the traumatic-theory concept.

288. Regeneration of Rabbit Submaxillary Gland Following Obstructive Adenitis.—*Surindar N. Bhaskar, Oral Tumors Branch, Armed Forces Institute of Pathology, Washington, D.C.* From both experimental and surgical material it has been established that duct obstruction leads to inflammation and atrophy of the salivary glands. The present study was undertaken to determine the regenerative ability of a salivary gland which had undergone experimental obstructive adenitis. For this purpose, in 20 adult rabbits the right and left submaxillary gland ducts were ligated. Seven of these were sacrificed at 7 days postoperatively. In the remaining, the ducts were again exposed and the obstruction eliminated. These 13 animals (26 glands) were sacrificed from 2 to 31 days after the removal of duct obstruction. The findings were (1) The control animals showed atrophy of almost all acini, appearance of undifferentiated epithelial cells, dilatation of major ducts, and infiltration of the interstitial tissue by plasma cells, lymphocytes, and neutrophils; (2) in all glands where the obstruction had been eliminated for 6 days or longer, there was evidence of varying degrees of regeneration of acini, a progressive decrease in inflammatory exudate, and appearance of secretory granules in the acinar cells; (3) at the maximal post-experimental period a complete regeneration of the gland was not demonstrated. It was, however, apparent that, following obstructive adenitis, if the duct blockage is promptly eliminated, the gland is capable of rapid regeneration.

289. Posterior Border Movements of the Human Mandible.—*Louis Boucher and Jay Jacoby, Marquette University, Milwaukee, Wisconsin.* The purpose of this investigation was to determine whether the posterior border movement of the mandibular condyles in the articular fossa are limited by ligaments or muscles. Maxillary and mandibular casts were made of the dentition of each patient and mounted in centric occlusion on an articulator. Acrylic resin clutches were fabricated to fit around the teeth and retain the tracing apparatus and central bearing pin. Lateral interferences

between the clutches posterior to centric occlusion were eliminated. Gothic-arch (stylus) tracings were made of the posterior border movements of twelve patients before, during, and after subjecting the patients to general anesthesia and curare. Photographs were made of each set of tracings. It was found that the condyles of the patients under anesthesia and curare could be moved posterior to the posterior border position. From this investigation we concluded that muscles were responsible for limiting posterior border movements of the condyles in the articular fossa of the human temporomandibular joint.

290. The Application of Electronic Instrumentation to the Practice of Dentistry.—*Jack L. Hartley and Walter V. Davis, School of Aviation Medicine, USAF Aerospace Medical Center, Brooks AFB, Texas.* A significant advance in the treatment of patients has been the application of small, relatively inexpensive electronic instruments for the determination of biophysical responses of the patient undergoing dental treatment. The instruments to be demonstrated and discussed are as follows: (1) A transistorized psychogalvanometer for indicating significant changes in skin resistance as a corollary "read-out" of autonomic nervous system activity. When coupled with a small transistorized amplifier with speaker, which converts any electric signal into a frequency-modulated sonic note for aural monitoring of the P.G.S.R. output, the instrument emits a changing tone as a continuous aural indication of patient anxiety and pain during routine restorative or surgical procedures. The instrument makes use of the psychogalvanic reflex to provide the dentist with a quantitative indication of patient stress, thus serving as a guide and adjunct to his technique in treatment. (2) A transistorized, compact, self-powered instrument consisting of a miniaturized photoelectric plethysmograph transducer and bridge amplifier for sensing and indicating, as an electrical signal, the cardiovascular pulse-pressure wave. It may be used peripherally or centrally as necessary, depending on placement site. When this is coupled with the instrument producing the sonic note, aural monitoring of the pulse rate, systolic-dystolic differentiation, and relative blood-pressure changes may be monitored during oral, surgical, or anesthesia procedures. By the use of these instruments, the dentist is far better informed of the autonomic or cardiovascular changes occurring during and/or as a result of treatment. These changes are audible and eliminate the necessity for glancing away from the patient or for transfer of information by another individual, with resultant delays.

291. Estimation of Examiner Error in Caries Diagnosis for Clinical Trials. —*J. E. Alman, S. Fleisch, and V. Lisanti, Institute for Stomatological Research, Sias Research Laboratories, Brooks Hospital, Brookline, Massachusetts.* The clinical trial of a new dentifrice depends on the comparison of the new caries increment between the dentifrice under test and the control. These increments typically turn on a small proportion of the teeth examined, perhaps less than 10 per cent. It seems intuitively obvious that examiner errors will not be randomly distributed over all tooth surfaces but will tend to concentrate among surfaces contributing to an increment. While the total number of errors may be small, considering the number of tooth surfaces inspected, it may be decidedly non-trivial relative to the number of surfaces contributing to the increments. This paper proposes some experimental designs for estimating examiner error rates, gives the mathematical model associated with each design, and gives examples of error-rate estimation from data. Two kinds of examiner errors are defined. Type I is the error of reporting a sound surface as carious, Type II is the opposite error. Within each type there are correlated and uncorrelated errors, and those that lie within and without an examiner's "zone of unreliability." Estimation of examiner error rate is illustrated from several sets of clinical data. An over-all index of examiner unreliability is proposed and illustrated. Implications for estimating the effect of examiner error upon the observed increments in a clinical trial are discussed, together with some areas for further research.

XXIV. BIOCHEMISTRY AND ENZYMOLOGY. MARCH 26, MORNING, SECTION A

292. ANAEROBIC GLYCOLYSIS IN BOVINE DENTAL PULP.—*Victor E. Walters and Alton K. Fisher, Department of Stomatology, College of Dentistry, State University of Iowa, Iowa City.* This report is concerned with measurements of acid production in tissue slices of dentinogenetically active and inactive pulp under anaerobic conditions. Studies were conducted by the Warburg method at 38° C. Acid producion was measured as microliters CO_2 liberated per milligram of dried tissue and expressed as $Q^{N_2}_{CO_2}$. Observations on both types of pulp included measurements of a series of samples for endogenous acid production, as well as three series of samples with a substrate of 0.1, 0.2, and 0.4 per cent glucose, respectively. Measurements were made at 6 points in a span of 140 minutes. In all instances 0.2 per cent glucose solution was added immediately following the 80-minute period of measurement. The initial mean endogenous $Q^{N_2}_{CO_2}$ for dentinogenetically active pulp was 0.66, which showed a decline until glucose was added. It increased to 0.71 during the following 40 minutes. When 0.1, 0.2, and 0.4 per cent glucose were present initially in the medium, the mean $Q^{N_2}_{CO_2}$, at 30 minutes were 2.70, 1.89, and 1.36, respectively. Thereafter the resultant acid-production curves, which were inversely related to glucose concentration, declined continuously in a somewhat asymptotic pattern. Acid production in quiescent pulp was low, and the responses to various concentrations of glucose were not clearly defined. This study suggests that glycolysis in pulp under continuing anaerobic conditions tends to be a self-limiting process.

293. THE EFFECT OF GLUCOSE ON THE RESPIRATION OF DENTINOGENETICALLY ACTIVE BOVINE DENTAL PULP.—*Christian Schwabe and Alton K. Fisher, Department of Stomatology, College of Dentistry, State University of Iowa, Iowa City.* An earlier study in our laboratory has shown that anaerobic glycolysis in dental pulp becomes a self-limiting process. This study was conducted on dental pulp slices which were divided so that half of each organ was subjected to endogenous respiratory measurements while the other half was observed in association with a substrate of 0.2 per cent glucose. In each 2-hour period the tissue samples were measured successively for oxygen consumption, carbon dioxide production, and anaerobic acid production. Five continuous cycles of these measurements at 38° C. were accomplished on 19 usable samples by the Warburg methods. Under endogenous conditions the Q_{O_2} fluctuated gently between 1.68 and 0.95 but remained above 1.20 for most of the time. The Q_{CO_2} remained in the vicinity of 1.0 or slightly below, yielding R.Q. values indicative of little or no carbohydrate utilization. Except for the initial determination of $Q^{N_2}_{CO_2}$ 0.76 after 2 hours in vitro, the $Q^{N_2}_{CO_2}$ remained below 0.40. Where the Krebs-Ringer–bicarbonate solution contained 0.2 per cent glucose, oxygen consumption remained below the endogenous values until after the glucose concentration was significantly reduced. The Q_{CO_2} remained consistently above the Q_{O_2}, indicating predominantly carbohydrate metabolism and also considerable acid production in the presence of oxygen. Under anaerobic conditions a maximum $Q^{N_2}_{CO_2}$ of 5.95 was observed after 4 hours in vitro, and it declined thereafter. This study indicates that dental pulp has a considerable capacity for acid production that is greatest in association with oxidative respiratory processes.

294. THE DIRECT OXIDATIVE PATHWAY OF CARBOHYDRATE METABOLISM IN BOVINE DENTAL PULP.—*Douglas O. deShazer, Department of Stomatology, College of Dentistry, State University of Iowa, Iowa City.* Previous studies in our laboratory have demonstrated a reasonably high respiratory activity in bovine pulp and have indicated that carbohydrate metabolism plays a significant role in this tissue, which has a high anaerobic glycolytic capacity. The presence of the direct oxidative pathway of carbohydrate metabolism—the hexosemonophosphate shunt—has been demonstrated in a variety of normal and pathologic mammalian tissues. A quantitative method for

the assay of the first two enzymes in this cycle, which serve to identify the shunt, has been developed by Glock and McLean and entails following spectrophotometrically the reduction of triphosphopyridine nucleotide at 340 μ. The present study has shown that both glucose-6-phosphate and 6-phosphogluconic dehydrogenase revealed a maximum at pH 8.50 in dentinogenetically active pulp, in contrast to pH 9.00 obtained by Glock and McLean for rat liver. The activity of this enzyme at pH 8.50 in pulp is 230 ± 8 units/gm at 30° C. This value is higher than that reported by others for bovine ovary and lower than for bovine adrenal cortex. The portion of carbohydrate metabolism in rat liver that follows the pentose shunt has been shown to be as high as 75 per cent, and it has been suggested that this cycle furnishes much of the triphosphopyridine nucleotide necessary for synthetic processes. Quantitative studies on glucose-6-phosphate dehydrogenase in pulp in relation to dentinogenesis are in progress.

295. Fractionation of Human Saliva I. The Dominant Metabolic Role of Mammalian Cell Fractions.—*Bertram Eichel, Institute of Stomatological Research, Sias Research Laboratories, Brooks Hospital, Brookline, Massachusetts.* Whole human saliva has been successfully fractionated. The separation of mammalian cells has been confirmed by direct microscopic count of epithelial cells and leukocytes. The separation of bacteria has been confirmed by total bacteria plate counts. Employing the Warburg respirometer, oxygen uptake, aerobic glucolysis, and anaerobic glucolysis activities were determined for active whole saliva and the following fractions prepared from the same whole saliva: (1) mammalian cells, (2) bacteria, and (3) 27,000 $\times g$. supernatant. Ninety to 100 per cent of the activities are recovered in unwashed mammalian cell fractions. When washed, the mammalian cell fractions exhibit 76–88 per cent of the activities of the whole saliva. Repeated washing did not result in lower oxygen uptake. The first mammalian cell wash supernatant yields only 7–12 per cent of the metabolic activities of the whole saliva. Bacterial fractions exhibit 16–34 per cent of the activities found in whole saliva. Leukocyte contamination of these bacterial preparations probably accounts for a part of the activities, and the bacteria contribute the remaining activity. 27,000 $\times g$. supernatant fractions do not yield measurable metabolic activities. Contrary to the belief that salivary metabolism reflects oral microflora metabolism, it is concluded that aerobic and anaerobic metabolism in active whole human saliva is associated with mammalian cells.

296. Conversion of Sucrose to Polysaccharide by Oral Samples.—*R. S. Manly and Joel A. Dain, Tufts University School of Dental Medicine and Thannhauser Laboratory, Boston Dispensary, Boston, Massachusetts.* The immersion of dental plaque or salivary sediment for a few minutes in sucrose solutions has a persistent effect on glycolysis, lasting for 2–6 hours. It seemed possible that this action might persist because the sugar is converted to a less diffusible form, such as polysaccharide. The first question was studied by using the Loewus improvement of the anthrone method for analysis of the water extract of salivary sediment which had been treated with a sucrose solution. The sucrose was removed by extraction with alcohol, leaving the remaining carbohydrate presumably in polymeric form. Alcohol-insoluble carbohydrate did not form when sediment was steeped in sucrose solution at 4° C. but was formed at room temperature and to a greater extent at 37° C. The rate of formation remained constant up to 2 hours. The nitrogen content of the purified samples amounted to 0.5 per cent or less, suggesting a polysaccharide. Formation was shown by the Dische-cysteine-carbazole test to be most rapid from sucrose, less from fructose, and a mixture with glucose, and least from glucose alone. The polysaccharide content and formation rate differed for sediment from different individuals.

297. Chromatographic Separation of Human Salivary Proteins.—*Solon A. Ellison and Irwin D. Mandel, Department of Microbiology, College of Physicians and Surgeons, and Division of Stomatology, School of Dental and Oral Surgery, Columbia University, New York City.* Analyses of human parotid saliva employing paper electro-

phoresis and immunologic gel-diffusion methods indicated the presence of several protein and glycoprotein constituents. In order to obtain amounts of these sufficient to permit their definitive characterization, studies were made of saliva by column chromatography on DEAE-cellulose, CM-cellulose, and hydroxylapatite. Several eluting systems were tested in conjunction with each ion exchanger. Yield and resolution of the salivary proteins were judged from electrophoretic and immunologic examination of the fractions obtained. Satisfactory separation of proteins was achieved with DEAE-cellulose, using several buffer systems. When glycinate buffer was used, the columns were loaded with saliva equilibrated with 0.002 M buffer, pH 9.5, and, after washing with this buffer, gradient elution was employed, the final glycine concentration being 0.2 M. The wash contained the basic proteins. Amylase and Y-globulin were eluted next, when the glycine concentration reached 0.015 M, and were followed by serum proteins as the molarity of the eluant approached 0.1 M. Comparable separations were obtained employing hydroxylapatite and eluting with phosphate buffers of pH 6.4, increasing in concentration by a gradient from 0.002 to 0.1 M, and subsequently adding 2.0 M NaCl to the 0.1 M buffer. In this instance, the order of elution was unrelated to the electrophoretic properties of the proteins. Preparations of salivary amylase obtained chromatographically by several methods were as active as the crystalline enzyme, but even the most active preparations retained a trace of Y-globulin, which could be detected immunologically.

298. Paper Electrophoresis of Saliva from Harvard Caries-susceptible and Caries-resistant Rats.—*E. A. Sweeney and J. H. Shaw, Harvard School of Dental Medicine, Boston.* Protein determinations of pilocarpine-stimulated whole mixed saliva from caries-resistant and caries-susceptible strains did not indicate a significant difference in protein level, although the saliva of the susceptible animals tended to have a slightly higher protein concentration, despite its appreciably lower viscosity, than saliva from the resistant strain. Whole saliva of caries-susceptible and resistant rats was electrophoresed, using Schleicher and Schuell 2043-A gl. paper with a Spinco model R system and stained with amido black. This method routinely revealed at least 9 major protein components, 5 of which have a mobility analogous to those of known components in patterns from rat serum: albumin, α_1-, α_2-, β-, and γ-globulin. A prealbumin zone may be composed of several variable, not readily separable, components. Two closely allied bands occur on the cathodal side of the starting line. Another major component has a mobility between serum β-globulin and serum α_2-globulin. Regular differences occurred in the relative concentration of 2 protein components of susceptible and resistant rats. Saliva from adults of both sexes of the susceptible strain contained 90 per cent more β-globulin and 50 per cent less of the most cathodally migrating component than did comparable resistant rats. Five components accounted for 77 per cent of salivary protein in the resistant and 73 per cent in the susceptible. These, expressed as a percentage of the total protein, correspond to: albumin, 19 per cent, 16 per cent; β-globulin, 11 per cent, 19 per cent; γ-globulin, 17 per cent, 15 per cent; the component with mobility intermediate between the position of α_2- and β-globulin, 20 per cent, 19 per cent; and the most cathodally migrating component, 10 per cent, 4 per cent, for caries-resistant and caries-susceptible rats, respectively.

299. Nitro BT Formazan Disclosure of DPNH Reductase, Lactic Dehydrogenase, Glucose-6-PO_4 Dehydrogenase in Human Gingiva.—*Haritun A. Shahrik and Bertram Eichel, Institute of Stomatological Research, Sias Research Laboratories, Brooks Hospital, Brookline, Massachusetts.* DPNH reductase (DPNHR), lactic dehydrogenase (LD), and glucose-6-PO_4 dehydrogenase (G6PD) have been disclosed in human gingiva, employing our modification of the nitro blue tetrazolium histochemical method. The distribution of formazan deposition for DPNHR and LD is similar to that previously described for succinic dehydrogenase (SD). In general, the reactions in the epithelium are most intense in the basal-cell layers and appear to diminish progressively toward the superficial epithelial layers. Ranking the enzyme activities with

respect to intensity and rate of formazan deposition, DPNH is most reactive, LD second, and SD least reactive. Like SD activity, DPNHR activity is intensified by the addition of cyanide. In contrast, LD activity is inhibited in the presence of cyanide. The distribution of formazan following G6PD disclosure shows a reversal of the pattern seen for SD, DPNHR, and LD. Formazan deposition is most intense in the stratum granulosum and adjacent deeper layers, and the reaction decreases progressively toward the basal-cell layer. G6PD disclosure is intensified in the presence of cyanide.

300. Application of the Method of Steepest Ascent to the Response Surface of Nitrate-Nitrite Reductase Activity in Salivary Sediment.—*Paul W. Goaz, Helen A. Biswell, and M. Clinton Miller, Dental Section, Oklahoma Medical Research Institute, Oklahoma City.* A study was made to determine the optimum conditions for, and interrelations of, temperature, pH, and concentration of electron donor on the rate of nitrate reduction by the nitrate-nitrite reductase systems in a pooled sample of salivary sediment. By varying the conditions of pH, temperature, and electron donor concentration (yeast extract), the minimum reduction time was established in four sets of sequential experiments. Employing the method of steepest ascent to the linear approximation of the response surface, the co-ordinates (levels of pH, temperature, and electron donor) of each succeeding set were predicted. By proper choice of additional experimental points around the co-ordinates of the fourth set, the property of rotatability was preserved, and a second-order approximation to the response surface was determined. This approach permitted the calculation of the response surface from a much smaller number of experiments than would have been necessary with a complete factorial experiment or a single-factor method. It also provides the opportunity for the recognition of possible interactions between the variables. While the three variables examined were found to influence the enzyme activity, temperature contributed the predominating influence above pH 6.0 and electron donor concentration of 0.7 μg/ml. Final solution of the response surface provided the optimum conditions of pH 6.98, 45.9° C., and 0.97 μg/ml of the electron donor. The basis for the construction of the design will be briefly discussed and the details of the statistical analysis illustrated by the use of data from the measurement of nitrate-nitrite reductase activity of salivary sediment.

301. Effect of Papain on Cartilaginous Growth Sites in Rat Skulls.—*Olli V. Ronning and James T. Irving, Forsyth Dental Infirmary, Boston, Massachusetts.* Previous investigators have shown that epiphyseal cartilages of long bones in young animals become reduced in thickness or even completely ossified after intravenous or subcutaneous injections of crude papain. It is suggested that this effect results from release of chondroitin sulfate from cartilage due to the action of papain on the protein to which the chondroitin sulfate is attached. In order to obtain information about the effect of papain on synchondroses of the sphenoid bone and on the mandibular condylar cartilage, 30 growing rats of the Wistar strain were injected daily with a 2 per cent solution of crude papain at a dose level of 100 mg. dry enzyme preparation per kilogram body weight during 8 days. Two rats were killed daily up to 8 days. The remaining animals were killed in pairs at 3-day intervals. The two synchondroses of the skull base seem to be affected similarly to the epiphyseal cartilage in long bones: narrowing of the cartilaginous plate, disturbance of the columnar arrangement of the cells, which tend to be smaller than normal, and eventually sealing off the epiphyseal cartilage with bone. In the condylar cartilage of the mandible no obvious changes were seen. It thus appears that growth cartilages differ in their sensitivity to papain. In other experiments it has been found that the fibular epiphyseal cartilage is much more resistant to the action of papain than that of tibia.

302. Hydroxyproline in Enamel.—*Leslie R. Burrows, University of Rochester, Rochester, New York.* Freshly extracted human teeth were freed of all carious lesions

and surface enamel and then sectioned into slabs approximately 0.5 mm. thick. Next the dentin, the inner third of the enamel, and all pits and fissures were removed with a bur under a dissecting microscope. The enamel samples were washed in detergent, rinsed, and dried. Four samples were prepared weighing 998, 1,025, 680, and 605 mg. The enamel samples thus prepared were decalcified in 0.46 M EDTA, pH 7.2 or 7.4 inside a dialysis bag immersed in 750 ml. of the same fluid for 1 week. The decalcified enamel was dialyzed against distilled water or phosphate buffer pH 7.4 for 1 week. The soluble and insoluble protein fractions were separated by high-speed centrifugation or by filtration through millipore filter, pore size 10 or 50 millimicrons. The proteins were hydrolyzed in 6 N HCl at 105° C. in sealed glass tubes for 18 hours. The hydrolyzates were then dried, washed twice with distilled water, and chromatographed by two-dimensional paper chromatography by means of methanol-water-pyridine, followed by phenol-water–acetic acid–EDTA. The papers were stained with ninhydrin and isitin according to the procedure of Kolor and Roberts (*Arch. Biochem. and Biophys.*, **70**:620, 1957). Hydroxyproline was present in both soluble and insoluble fractions, but the amount present in the soluble fraction was very small.

303. Detection of Trace Constituents in Oral Fluids by Gas Chromatography.—*T. B. Weber and J. W. Rhoades, School of Aviation Medicine, USAF Aerospace Medical Center, Brooks AFB, Texas.* A vapor fractometer with a thermal conductivity detector was employed to assay at body temperature the volatile materials isolated from fluids in the mouth. Fresh samples of parotid fluid, whole saliva, and 1×10^{-4} M hexachlorophene rinse were stripped of organics by reduced pressure, and these volatiles were swept with helium carrier gas into a short preparative column. This column contained firebrick coated with a glycol derivative which effectively removed the excess moisture and carbon dioxide from the organic fraction. The unknown materials were then collected in a freeze-out trap and passed into the gas column. On the basis of retention times of known compounds passed into the system, tentative identifications were made on the materials found in the oral fluids. The 2-meter-long, Carbowax glass column provided a 40-minute detection period between the initial air peak and the water passage, which precluded further identification. In the parotid secretion four major components, all less than 1 ppm, appeared; whole saliva showed five easily delineated fractions even after an early, transitory masking of the chromatogram by ammonia; the hexachlorophene rinse showed five materials. It was possible to make quantitative determinations of the amounts present. Three peaks—acetone, methanol, and ethanol—were evident in all samples. Other fractions appeared to be in the p.p.b. range, and efforts are being made for their identification with a flame ionization detector.

304. The Collagen Content of Caries Susceptible Rat Tooth Germs.—*Pat O'Day and Lucien A. Bavetta, Department of Dental Biochemistry, University of Southern California, Los Angeles.* Numerous investigators have demonstrated a direct relationship between sucrose and dental caries. Since a high sucrose diet will produce appreciably more caries susceptibility than stock ration, and, since collagen is an important part of the organic matrix of teeth, it was of interest to compare the collagen content of the molars of rats on the two diets. After weaning, 16 litters of rats were divided, and half were placed on a 70 per cent sucrose diet and the other half on stock ration. One week after weaning, the animals were sacrificed, and the collagen content of the different molars was determined by a modification of the Neuman and Logan hydroxyproline method. A significantly higher ($p = 0.001$) amount of collagen was found in the unerupted third molars of the sucrose group in comparison with the stock-fed group. The third molars of the sugar group contained 10 per cent more collagen than did the stock group. Data also show that the teeth of the stock group contained significantly greater amounts of hexosamine than the sucrose group and that the incorporation of labeled glycine into tooth collagen was significantly greater in the sucrose-fed group. The organic matrix of teeth is made up of collagen and ground

substance; thus the alteration of this moiety might possibly lead to a more caries-susceptible tooth which could be more readily damaged after maturity.

XXV. CALCULUS AND PLAQUE FORMATION. MARCH 26, MORNING, SECTION B

305. THE HISTOLOGY OF THE RESPONSE TO ULTRASONIC CURETTAGE.—*Leo Zach, Department of Pathology, New York University College of Dentistry, New York, and Gerson Cohen, Dental Department, Veterans Administration Hospital, New York.* An investigation of the comparative response of subgingival tissues to hand-instrumented and ultrasonic scaling was undertaken to determine whether either technique would reveal histologic evidence of advantage over the other. The following factors were considered significant as potentially desirable results in scaling and are compared: (*a*) absence of inflammatory exudate, (*b*) retention of polymerization of transseptal fibers, (*c*) rapidity of healing, (*d*) ability to "strip" epithelium from tissue side of sulcus. Three mature *Macacus rhesus* monkeys with markedly worn teeth and periodontosis were hand-scaled in one quadrant and ultrasonically scaled in two quadrants, with one quadrant left as control. Results demonstrate histologic evidence of the possibility of selectively stripping epithelium from the sulcus with the ultrasonic instrument. Ultrasonic scaling results in somewhat more pronounced disruption of uppermost peridental fibers, with, however, a lessened inflammatory response in subjacent tissues. Recovery to healing seems accelerated as observed in 2-day and 1-week specimens. The significant difference between hand and ultrasonic scaling, if peeling the epithelium from a pocket wall is considered therapeutic, lies with the ability of the ultrasonic vibrator to perform this function. Localization of tissue response to sharply delimited areas and a rapid dissipation of effect at a short distance from instrument application indicate the possibility of performing highly selective procedures, such as "microcauterization," in the pocket area.

306. CARBOHYDRATE CLEARANCE FROM TOOTH INTERPROXIMAL SPACES.—*Joel Goldsby, Theodore Koulourides, and Joseph Volker, University of Alabama Medical Center, Birmingham.* Information pertinent to the retention and clearance of carbohydrates from the interproximal areas of teeth has significance in the etiology of dental caries. The present study was undertaken to add to our knowledge of the subject. Removable dentures with natural teeth were constructed for five experimental subjects. After the volume of the interproximal space was determined, carbohydrate mixed with known amounts of water or saliva was placed in the area, and the dentures were inserted in the mouth. At varying intervals, the appliances were removed and cleaned except for the interproximal spaces, which were flushed with water. The washings were analyzed for carbohydrate with a modified anthrone method. The results of the analyses were expressed in glucose equivalents. Illustrative of our observations is the finding that an interproximal space containing 8 mg. of glucose equivalents at the time of denture insertion has only 100 μg. of glucose equivalents after 15 minutes. Similar findings have been observed in subjects wearing molar crowns with removable interproximal enamel inserts. When saliva is substituted for water as the mixture fluid, carbohydrate clearance is accelerated. These studies are basic to subsequent investigations on the effect of fat additions, etc., on interproximal carbohydrate clearance.

307. EFFECT OF DIFFERENT UREA CONCENTRATIONS ON PLAQUE pH IN VIVO.—*I. Kleinberg, Department of Biochemistry, University of Manitoba, Winnipeg, Canada.* Since in the fasting (i.e., prebreakfast) mouth, almost invariably dental plaque pH levels were found to be higher than the pH of the saliva washing them, it was therefore postulated that base was produced from salivary nitrogenous material by plaque bacteria faster than it could be removed (I. Kleinberg, *Biochem. J.*, **66**:61P, 1957). Since urea is susceptible to rapid breakdown, the present investigation was undertaken to determine how different urea levels could affect plaque pH's in situ. The pH was measured with antimony microelectrodes (I. Kleinberg, *Brit. D. J.*,

104:197, 1958), and the experimental design was similar to previous studies with glucose (I. Kleinberg, *J. D. Res.*, in press). When urea was added continuously over a period, the fasting steady-state pH was altered to a higher level; this level was directly related to the concentration of urea added, with a maximum at a concentration of approximately 2.0 per cent. When the urea concentration was allowed to decrease from various initial levels, the plaque pH rose at first and then fell as the urea concentration progressively decreased. If, during this fall, the urea level remained above a certain critical level for a long enough time, then the pH maximum could be reached. Low concentrations could induce a relatively large pH rise, so that salivary urea levels (G. Nikiforuk *et al., J. Pediat.*, **49**:425, 1956) might account for the difference in the fasting plaque and saliva pH levels mentioned above.

308. The Carbohydrate Components of Supragingival Calculus.—*Berge Hampar, Irwin D. Mandel, and Solon A. Ellison, School of Dental and Oral Surgery and College of Physicians and Surgeons, Columbia University, New York.* Pooled samples of supra-gingival calculus collected from the lingual surfaces of lower anterior teeth were dried in a desiccator over P_2O_5. They were placed in dialysis sacs, demineralized by dialysis against 5 per cent disodium ethylenediamine tetracetate adjusted to pH 7.8, and then dialyzed against water. The non-dialyzable residue (matrix) was lyophilized and weighed and was found to constitute 9–17 per cent (average 13 per cent) of the initial sample. Twenty-five per cent of this matrix was soluble in water. Samples representing either total matrix, insoluble matrix, or soluble matrix were hydrolyzed with Permutit Q,* 0.1 N HCl, or 2 N HCl, and then assayed for hexoses, fucose, and hexosamines. After hydrolysis, neutral sugars were recovered by passage through Amberlite M-B3,† and then identified by paper chromatography, using propanol: ethonal: water: (7:1:2) as the solvent and aniline hydrogen phthalate as the developer. It was found that the total matrix contained 6 per cent hexoses, 4 per cent fucose, and at least 2 per cent hexosamines. The hexoses and fucose were almost equally divided between the soluble and the insoluble matrix. On chromatograms the neutral sugars appeared to be glucose, galactose, mannose, fucose, rhamnose, and either xylose or arabinose.

309. The Effect of Local Environmental Changes on Calculus Formation.—*Samuel Turesky, Grete Renstrup, and Irving Glickman, School of Dental Medicine, Tufts University, Boston, Massachusetts.* A histologic and histochemical study was conducted to determine the manner in which the progress of calculus formation was affected by changing the oral environment. Two-day plaques collected on cellulose acetate strips were interchanged between the mouths of calculus formers and non-calculus formers. Two-day and 7-day specimens, which were not transferred but remained in situ, served as controls. Microscopic sections were stained with hematoxylin and eosin, toluidin blue, and von Kossa's silver technique. Placing 2-day strip specimens from a non-calculus former in the mouth of a calculus former resulted in more plaque accumulation and a greater degree of calcification than occurred on controlstrips with were not transferred. In specimens changed from a calculus former to a non-calculus former, plaque accumulation and calcification was less than that which occurred on strips that were not transferred. In some instances the calculus which accumulated on the transferred strip was greater in amount than that which ordinarily occurred on the non-calculus former. The finding suggests that the rate of calculus formation is affected by a change in the salivary environment and that calculus formation can be stimulated by the presence of a local "seeding" mechanism in a mouth where it ordinarily occurs in slight or negligible amounts.

* The Permutit Co., New York.

† Rohm & Hass, Philadelphia, Pennsylvania.

310. THE CHEMISTRY OF ORAL CALCULUS FORMATION.—*Duncan McConnell and Walter J. Frajola, Health Center, Ohio State University, Columbus.* Under experimental conditions (temperature, pH, concentrations, etc.) which simulate a physiologic environment, it is possible to obtain heavy deposits of in vitro calculus from boiled saliva by adding small quantities of carbonic anhydrase. When a known inhibitor of the enzyme is also added, the deposition does not occur. Comparable experiments with synthetic solutions demonstrate that "in vitro bone mineral" requires merely three inorganic ions (calcium phosphate and carbonate) and a single organic catalyst (carbonic anhydrase). As a result of these and related experiments, we conclude that the precipitate which forms is a carbonate hydroxyapatite (dahllite), that the presence of carbonate ions is essential to calculus formation, that the reaction can be inhibited by inactivation of the enzyme, and that carbonic anhydrase almost certainly plays a fundamental role in vivo.

311. THE ORGANIC MATRIX OF DENTAL CALCULUS.—*M. F. Little, C. Casciani, and S. Lensky, Eastman Dental Dispensary, Rochester, New York.* Following EDTA demineralization of supra-gingival calculus, a matrix was recovered which invariably retained the volume and configuration of the original sample. In some instances the matrix appeared as a fragile, filamentous meshwork. However, the majority differed from the original calculus only in the development of a translucent appearance and a firm pliability. After dialysis and lyophilization, the matrix accounted for 16–28 per cent of the original wet weight of the calculus. The matrix lost between 90 and 99 per cent of its weight on ashing; the ash had a Ca:P ratio of 1:21. Carbohydrate, as assessed by anthrone, accounted for 12–20 per cent of the matrix, while nitrogenous components, as calculated from micro-Kjeldahls, accounted for 36–40 per cent. The presence of 21 amino acids and 7 sugars was tested in hydrolyzates of the matrix, using specific reagents and paper chromatography with suitable solvent and indicator systems, for positive qualitative identification. The results indicated that 18 amino acids were consistently present in all the hydrolyzates, with half in relatively high concentrations. Hydroxyproline, leucine, and methionine appear to be absent. To date, among the sugars, galactose and ribose, as well as an unidentified non-running pentose, have been found.

312. MICRO pH ELECTRODES FOR ORAL PLAQUE OR OTHER VISCOUS SOLUTIONS.—*John J. Parran, Procter & Gamble Co., Ivorydale Technical Center, Cincinnati, Ohio.* Electrodes suitable for measuring the pH of microquantities of oral plaque were constructed, by means of a commercially available glass electrode. The principle used was that of spreading the plaque into a thin film between the sensitive surface of the glass electrode and the tip of the reference electrode. A fiber-type reference electrode tip was molded to fit the glass electrode by making a direct cast of the glass electrode tip with chemically activated acrylic resin. Polypropylene was used to construct the remainder of the reference electrode. The electrodes were incorporated into an assembly designed so that depressing a syringe would fit the reference electrode tip to the glass electrode by a simple hydraulic mechanism. The electrodes have proved to be rugged and capable of measuring the pH of small quantities of plaque with good accuracy.

313. CHANGES IN PLAQUE pH FOLLOWING GUM CHEWING AND TOOTH BRUSHING.—*John W. Hein, Pramod M. Soparkar, and Gertrude A. Quigley, School of Dental Medicine, Tufts University, Boston, Massachusetts.* Both glass and antimony electrodes were used to measure the effect of gum chewing and tooth brushing on the pH of dental plaques following 25 per cent glucose rinses. Two groups of six subjects abstained from regular oral hygiene procedures from 3 days prior to tests to the end of each series of experiments. Two interproximal and one smooth-surface pH readings were taken in both maxillary quadrants. Chewing (10 minutes) and brushing (30

seconds) were restricted to the right quadrant. Readings were taken in most cases at 10-minute intervals. Brushing with and without water and by three brushing techniques were investigated. While pH values showed considerable variation by surface between subjects and within the same individual on different days, the following conclusions are supported by marked statistical significance. Brushing per se, with or without water, had little effect on plaque pH. In most subjects the chewing of gum* gave a marked, rapid, and sustained rise in pH.

314. Some Leukocyte-Plaque Relationships.—*J. D. Wildman, Microbiological and Biochemical Center, Syracuse University Research Corporation, Syracuse, New York.* The leukocytes taken up with specimens of soft plaque have been shown to become migratory in Ringer's solution modified by the addition of sodium hexametaphosphate. Other substances also activate the leukocytes. On the other hand, relatively few plaque specimens mounted in saliva alone have shown leukocyte activity. Of special interest is the fact that dry sucrose added to saliva has resulted in migratory activity of leukocytes, indicating that the spherical conditions usually observed when plaque is mounted in saliva may not be due to any specific inhibitory activity of the saliva itself. Of possible importance from the standpoint of plaque control have been the observations of whole blocks of organisms apparently engulfed by activated leukocytes in a mass phagocytic phenomenon. It has previously been shown that leukocytes may be attracted to the amorphous bacteria-laden gel material in plaque and appear to penetrate such material. Further studies with time-lapse methods have shown that they may, in fact, enter loose plaque and move around. They may continue active movement within the debris, become stationary, or move outward. Small particles, possibly derived from leukocytes, have been observed in activity, and one instance such a particle was seen to be picked from a hyphal strand by a leukocyte. A very interesting observation has been that of an activated migratory leukocyte moving inside a clear spherical area within a squamous-type cell. Sudden changes have been observed with time-lapse, which, in the course of ordinary microscopic observation, would probably not be noticed.

315. Formation and Properties of Organic Films on Teeth.—*A. H. Meckel, Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio.* Organic films covering the enamel surface are usually identified as Nasmyth's membrane or as secondary enamel cuticle. The persistence of the former after eruption of the tooth is generally regarded as questionable. The possibility of a relationship between the secondary cuticle and saliva has been mentioned in the literature, but evidence for this concept is still scarce. Experiments with cleaned enamel surfaces which were inserted into partial dentures and held in the mouth for various lengths of time showed that an organic film can be recovered from such enamel within a day. This renewable film or pellicle appears to be identical with published descriptions of the controversial secondary enamel cuticle. This pellicle withstands average tooth brushing; it is transparent, usually colorless, and practically free of bacteria. The film is 2–4 mμ thick, and its presence can often be proved only by dissolving the underlying enamel and floating off the film. Dental plaque sometimes covers a part or all of its surface. By incubating enamel in saliva it has been possible to form artificial pellicles which physically and histochemically closely resemble the organic films formed on enamel in the mouth. Histochemical staining reactions were very similar for pellicles and salivary films of a variety of known origins. When any of the films under investigation were treated with various enzymes, only pepsin caused histochemical or physical changes. The possible presence of an organic film which may act as a diffusion barrier on the enamel surface must be taken into account when studying the solubility or reactions of enamel.

* Dentyne chewing gum.

316. Interrelated Effects of Diabetes, Arteriosclerosis, and Calculus on Alveolar-Bone Loss.—*Richard S. Mackenzie and H. Dean Millard, University of Michigan School of Dentistry, Ann Arbor.* Does the diabetic individual lose more alveolar bone than the non-diabetic when certain other factors are considered? To explore this question, the extent of alveolar bone loss, tooth loss, presence of calculus, and the association of alveolar bone loss with calculus were compared in three experimental and control groups of examinees from the Faculty Health Appraisal Program at the University of Michigan. The three experimental groups consisted of 60 diabetics, including 24 arteriosclerotics; 64 diabetic suspects, including 15 arteriosclerotics; 54 arteriosclerotic non-diabetics. Their controls consisted of 92 non-arteriosclerotic, non-diabetic examinees. The groups were classified by means of urinary tests, blood glucose levels, and glucose tolerance tests. The designation "arteriosclerotic" was made on the basis of retinal arterial changes. The individuals in the experimental groups were selected for the study only if they had no diagnoses of other metabolic disease. Their controls were selected in an unbiased manner to match them as to sex, race, age, and absence of any diagnosed metabolic disease. Alveolar bone loss, tooth loss, and prevalence of interproximal calculus were determined radiographically. The findings were as follows: None of the groups showed a significant tendency to have more alveolar bone loss, tooth loss, or calculus than controls. Each group showed a statistically significant tendency ($P < .001$) for a tooth surface bearing calculus to be associated with increased loss of the alveolar bone adjacent to that surface. But none of the experimental groups showed the finding of increased alveolar bone loss associated with calculus to an extent significantly different from their controls.

XXVI. PULP STUDIES AND PROBLEMS RELATED TO STRESS. MARCH 26, MORNING, SECTION C

317. Repair of Periapical Lesions Following Pulp Capping of Vital Teeth.—*John Sapone, University of California School of Dentistry, San Francisco.* In the years 1955–60 the University of California initiated, by utilizing the numerous cases available in the clinic, a clinical test to evaluate the effect of direct pulp capping with calcium hydroxide through a standardized capping procedure. Through these tests it was revealed roentgenographically that repair of periapical lesions has occurred on some teeth. The procedures used were (1) teeth selected with requisites of vital pulp capping; (2) electric and thermal vitality tests made; (3) rubber dam used; (4) all clinical caries removed; (5) hemorrhagic exposure produced prior to capping; (6) a tissue-compatible antibacterial solution, such as 9-aminoacridine, applied for 5 minutes with a sterile cotton pledget; (7) a calcium hydroxide capping material (calcium hydroxide 65 per cent plus barium sulfate 35 per cent added as a roentgenographic contrast media) applied over the exposure site and sealed in place with a layer of zinc oxide and eugenol, followed by a zinc phosphate cement base. Five hundred and forty-two completed pulp-capping cases have been accumulated for study between the years 1955 and 1960. In 6 of these cases the pulp was extirpated. In 16 the tooth was extracted. Therefore, 520 clinic cases may be considered successful. Of these cases, 118 roentgenographically showed clearly some periapical lesion or involvement existed prior to, or at the time of, the pulp capping. A follow-up recall now in progress reveals that a repair of the periapical lesion has occurred in at least 13 out of 15 cases examined. This study is being continued in order to bring forth additional supporting evidence to the findings.

318. Pulpal Response to Increased Rotational Speed.—*Alfred Schuchard, University of California School of Dentistry, San Francisco.* Are the reported changes which take place following the use of high rotational speeds in cavity preparation reversible or non-reversible? Clinical experience seems to indicate that the changes

produced are reversible. Irritation or stimulation in any part of the body indicates a response of defense by cellular components in that area. The irritation produced in the pulp by grinding procedures in teeth is no exception. Permanent bicuspids from four patients, aged twelve and a half to twenty-one years, were used in this series. All teeth were clinically and radiographically asymptomatic, with no history of pulp damage. The preparations (Class II or M.O.D.) were produced by using a belt-driven instrument rotating at 160,000 r.p.m. All work was carried out with the rubber dam in place, using air alone as a coolant. The teeth were restored with amalgam. The first tooth was extracted 1½ hours after placement of the restoration. The remaining four teeth were extracted at periods of 2, 8, 13, and 31 days. Immediately after extraction, the teeth were fixed, subsequently decalcified, and serial sections 10–12 mμ thick obtained. Representative sections are presented. This study indicates that loss and upset of the odontoblastic layer is the best indication of response. Within 2 weeks, and definitely within 31 days, recovery was apparent.

319. (Not presented.)

320. Clinical Evaluation of Indirect Pulp Capping: Progress Report.—*Joseph J. Damele, University of California School of Dentistry, San Francisco.* A method for treating deep carious lesions intended to circumvent pulpal exposure has been introduced. A phenomenon which has recently been described as "reconstructed dentin" is the rationale for its use. The purpose of this project was to test its effectiveness clinically, to compile data on each case, and to obtain, where ethically possible, specimens for histologic examination. Teeth treated were those displaying carious lesions, warranting strong suspicion of pulpal involvement. To date, in 70 cases the treatment has consisted of incomplete caries removal, the placement of a bulk of calcium hydroxide, and an alloy temporary. After a period of about 60 days the calcium hydroxide and all clinical caries were removed. The data acquired on each case include preoperative roentgenograms, electrical, thermal, and percussion tests; subjective symptoms; and postoperative roentgenograms. Two months later the testing procedure was repeated after careful, but complete, caries removal. The operator rendered a prognosis, giving reasons. A permanent restoration was placed, and 6 months later all the above tests were repeated. To date, of the 70 cases thus treated, roentgenographic evidence indicates increased radiopacity adjacent to the pulpal wall in the dentin in 90 per cent of the cases. Pulpal exposure was unnecessary in 85 per cent of the cases, and all the teeth thus far treated have remained asymptomatic and vital.

321. Electron Microscopy of the Dental Pulp.—*M. B. Quigley, University of Alabama Medical Center, Birmingham.* Although the ultra-structure of the odontoblast has been described, the remainder of the pulp has received little attention. Incisor and molar teeth of hamsters and the molars of dogs were removed, and the pulp immediately freed from the dentin walls, cut into small pieces, and fixed in buffered osmic acid, dehydrated, methacrylate-imbedded, and sectioned for electron microscopy. The cells of the pulp were observed to be round or oval with a relatively large nucleus, with a nucleolus present in most cells. There was scant endoplasmic reticulum and few mitochondria. A fat droplet was observed in most cells and, when present, was usually found at one pole of the nucleus. There were many minute protoplasmic processes extending out from the cell. In some instances, a syncytial-like arrangement of the cells appeared, but a cell membrane could always be traced between the cells. Collagen could not be demonstrated in the intercellular areas. Instead, small filamentous strands randomly arranged were seen. The majority were of the order of 25 A by 2000 A. However, it must be borne in mind that sectioning was not always in the long axis of the filaments. This intercellular material closely resembled tropocollagen. More pulps were prepared for electron microscopy, using chilled acetone and ethyl chloride as a fixative. This technique did not preserve the cells, but the

extracellular filaments in this instance were much wider, suggesting a lateral condensation of the fine filaments which had been seen in osmic acid–fixed material.

322. PULPAL RESPONSE OF VITAMIN A–DEFICIENT RATS TO OPERATIVE PROCEDURES.—*Clive I. Mohammed and Dorothy F. Mardfin, University of Puerto Rico School of Dentistry, San Juan.*—This study is based on complete serial sections through the mandibular incisors of 80 albino rats. Thirty-five animals were fed a basal vitamin A–free diet. In all cases pair-fed controls receiving 400 U.S.P. units of vitamin A weekly were maintained. An additional 10 animals served as ad libitum controls. When weight curves showed a downward trend, cavities were prepared in the left mandibular incisors and were filled with zinc oxide and eugenol. The animals were sacrificed at intervals varying from 1 to 7 days. The severity of the deficiency varied in different animals, as evidenced by the response of the incisors and correlated closely to the weight curves. In all cases the labial (enamel-covered) odontoblasts were different both morphologically and in staining reaction from those of the lingual (cementum-covered) odontoblasts. In the most severe deficiencies the lingual odontoblasts appeared as small, flattened cells not essentially different from other pulpal cells. The labial odontoblasts were columnar and appeared normal. The lingual dentin was abnormally thin, essentially atubular, and did not stain with azure B. The labial dentin, although thick and with regular tubules, appeared to be interglobular dentin. Labial odontoblasts responded to the operative procedure by the apposition of dentin, which was comparable to the response seen in the pair-fed and ad libitum controls. The lingual odontoblasts, however, showed no response to the cavity preparation.

323. THE EFFECTS OF ANTARCTIC STRESS ON VITAMIN REQUIREMENTS OF MAN.—*Robert J. Adams and William R. Stanmeyer, U.S. Naval Medical Research Laboratory, New London, Connecticut, and Richard S. Harding, U.S. Army Medical Research and Nutrition Laboratory, Denver, Colorado.* According to previous Antarctic expedition reports, tooth mobility, painful bleeding oral tissues, extreme weakness, and other physical disorders encountered were suggestive of vitamin B and C deficiencies. The experimental purpose of this work was to determine man's reaction to the stresses of the Antarctic environment by observing oral health and vitamin requirements. These factors were measured monthly, for 8 consecutive months, in an experimental group of eleven males by a vitamin tolerance test (2-cc. intramuscular single injection of thiamin, 5 mg.; riboflavin 5 mg.; niacinamide, 100 mg.; and sodium ascorbate, 300 mg.) and an oral examination. Fasting urine specimens and 9-hour test-loaded specimens were collected, acidified, stored, returned to the United States, and analyzed to determine vitamin concentrations. In a temperate climate, a control study duplicating storage conditions was repeated, and vitamin deterioration rates of Antarctic specimens were established. Antarctic test results indicated that excretion levels did not fall below the range of normal values in either the fasting or the load states. Observations suggested that fasting ascorbic acid levels had a tendency toward depletion but were not indicative of deficiency at the time of experimental conclusion. Oral mucous membrane ulcerations, dryness of the mouth, cracking of the lips, and small oral hematomas were observed. It is suggested that decreased ascorbic acid levels and other marginal vitamin insufficiencies were not the single causes of oral disease but probably played a most influential supporting part in conjunction with other factors of climatic and psychological stresses.

324. STRESS PATTERNS IN DENTAL PATIENTS: EFFECT OF LOCAL ANESTHETIC INJECTIONS.—*Warren R. Hester, John R. Prigmore, and Ira L. Shannon, School of Aviation Medicine, USAF Aerospace Medical Center, Brooks AFB, Texas.* To provide base lines for concurrent stress evaluations in oral surgical procedures, this study was designed to answer the following questions: (1) Does the administration of local anes-

thetic agents in the oral area produce detectable adrenocortical response? (2) If so, is there a difference in response between commonly employed agents? (3) Is the site of injection of significance in this regard? Pre- and postinjection blood samples were drawn from 61 subjects, 41 of whom received maxillary buccal infiltration, 20 received Xylocaine,* and 21 were injected with Ravocaine.† The remaining 20 subjects were anesthetized as for the removal of a lower molar tooth. None of the subjects was aware of his impending participation or of the fact that a second blood sample was to be drawn. Specimens were analyzed for 17-hydroxycorticosteroids, sodium, and potassium. Pre- and postinjection steroid means were as follows: maxillary Ravocaine, 14.63 (S.D., 3.56) and 14.96 (S.D., 3.10); maxillary Xylocaine, 14.92 (S.D., 3.17) and 14.44 (S.D., 2.74); mandibular block, 12.38 (S.D., 3.85) and 13.97 (S.D., 3.98) gamma per cent. None of these differences was significant and no differences were found for sodium and potassium. Thus all three questions posed may be answered in the negative.

325. Stress Patterns in Dental Patients: The Adrenocortical Response to a Scheduled Appointment for Exodontia.—*Ira L. Shannon, John R. Prigmore, and Gerald M. Isbell, School of Aviation Medicine, USAF Aerospace Medical Center, Brooks AFB, Texas.* Serum free 17-hydrocorticosteroids (17-OHCS), sodium, and potassium were determined in 114 young male adults. In 61 control subjects 8:00 A.M. means of 13.88 gamma per cent, 143.4 mEq/l, and 4.69 mEq/l, were found for steroids, sodium, and potassium, respectively. Comparable means for 53 subjects tested prior to undergoing exodontia were 18.08 gamma per cent, 142.5 mEq/l, and 4.71 mEq/l. The 17-OHCS difference between the two groups was significant ($P < 0.01$), and the anticipation of the oral surgery was held responsible therefor. All operated subjects had known for at least 48 hours of the time of the exodontia appointment. At 11 o'clock the control group steroid mean was 14.46 gamma per cent, while that for the experimental subjects 3 hours after exodontia was 16.28 gamma per cent. Thus the significantly high starting level in the surgery patients masked the adrenocortical stimulus, if any, resulting from the operative procedure. Results will also be presented on 300 additional subjects operated under varying time schedules and, in many instances, without prior knowledge of the impending operation.

326. Emergency Dental Care for Nuclear Submarine Personnel.—*A. G. Nielsen, Dental Branch, U.S. Naval Medical Research Laboratory, U.S. Naval Submarine Base, New London, Connecticut.* Reviews are presented of dental emergencies recorded in 1,500 World War II submarine patrol reports, as well as dental emergencies reported in response to a questionnaire distributed to currently operating submarines in the Atlantic Fleet. The advent of nuclear submarines has expanded the emergency dental-care problem for personnel removed from dental facilities for extended periods. A suggested program to meet the dental needs of such personnel is presented. The program includes preventive measures to reduce the occurrence of dental emergencies under way and a training program or medical department personnel to provide emergency care for unpredictable dental problems. A suggested modification of the initial outfitting list of dental supplies and equipment for nuclear submarines is presented. Included in this list is a new portable dental air turbine complete with two handpieces, aspirator, air and water syringes, handpiece light attachment, and foot control. The portable unit is 10″ wide, 9″ high, 12″ long, and weighs 19 pounds. This unit was developed specifically for submarine use. It is powered directly from the submarine air-pressure system. The role of the portable unit for submarine emergency dental care is discussed.

327. Facial Protective Device for Personnel Wintering-Over in Antarctica.—*A. G. Nielsen and M. J. Perlitsh, Dental Branch, U.S. Naval Medical Re-*

* Astra Pharmaceutical Products, Inc., Worcester, Massachusetts.

† Cook-Waite Laboratories, Inc., New York City.

search Laboratory, U.S. Naval Submarine Base, New London, Connecticut. A review of Antarctic facial-protection problems is presented, and a prototype face mask is described which has enabled the wearer to be comfortable for 1 hour in a cold test chamber at —70° F., as against extreme discomfort experienced after 5 minutes under identical conditions but without the mask. The mask is light-weight and provides protection for the face, eyes, oral structures, and the respiratory tree. The mask has no external power source but relies entirely on recycling of natural body energy (heat and moisture). With the mask in place, the temperature of the inhaled air measured on the labial surfaces of the central incisors is 60° F. Without mask protection, temperatures measured under identical conditions were as low as 32° F. The eye protective device remained fog-free for the 1-hour test period. Ice accumulation within the mask was not a problem during the test period. Provisions have been made for protection against snow blindness. The mask is currently being field-tested in Antarctica, and it is hoped that it will provide protection for the previously mentioned structures, reduce the desiccation of exposed tissues and the consequent loss of body moisture, and also reduce fatigue through the conservation of energy ordinarily lost in the exhaled air.

XXVII. ORAL BIOLOGY II. MARCH 26, MORNING, SECTION D

328. (Not presented.)

329. INTERRELATIONSHIPS OF HUMAN SALIVARY FLOW RATES FROM VARIOUS STIMULI.—*Gerald J. Cox, School of Dentistry, University of Pittsburgh, Pennsylvania.* Rate of flow of whole saliva was determined by weight per hour in 92 young adults. Chewing stimuli were paraffin, unflavored chicle, and rubber bands. Sucking stimuli were pieces of hard candy with three levels of content of citric acid and no acid. The data were organized in increasing weight of mean resting saliva. The weight of flow was analyzed by association with history of mumps, choice by taste of the acid candies, neutralization of acid by the saliva, time of clearance of glucose from the mouth, time of appearance of ingested iodide in the saliva, and dental caries experience. Analyses of the data were on (1) variation for the individual with respect to stimuli and (2) comparison among subjects.

330. CHEMICAL ASPECTS OF GINGIVAL DISTURBANCES.—*Ingeborg Constantinos and B. R. Bhussry, School of Dentistry, Georgetown University, Washington, D.C.* This investigation represents an effort to characterize some of the clinical changes in various forms of gingival disturbances by means of chemical techniques. The gingival tissue for this project was obtained from patients in the Department of Periodontia. Patient history, clinical examinations, and full-mouth X-ray were obtained. The patient was examined and diagnosis made. Color photographs* were made to maintain a record of the clinical appearance of the gingival tissue prior to biopsy. The gingival biopsy was taken, and the tissue was washed in normal saline to remove blood and saliva. These tissues were subjected to chemical analyses. An estimate of the concentration of three major components of gingival connective tissue—hexosamine (ground substance), hydroxyproline (collagen), and nitrogen (tissue protein)—was made. Results obtained were compared with similar data from clinically normal gingival tissue. Although the hexosamine content did not show any appreciable differences between the normal and inflamed gingiva, alterations in the hyroxyproline were evident. This suggests changes primarily in the collagen component of gingival connective tissue during inflammation.

331. (Not presented.)

332. SYMBOL RECOGNITION IN SCIENTIFIC COMMUNICATION.—*Walter V. Davis, Jack L. Hartley, Warren R. Hester, and Douglas H. Hagen, School of Aviation Medicine, USAF Aerospace Medical Center, Brooks AFB, Texas.* Eighty dentists and lay-

* Kodachrome.

men were tested on their knowledge of statistical symbols, phrases, and formulas used in 16 issues of the *Journal of the American Dental Association*. The individuals examined represented a wide range of age, education, and experience level in a specific vocation. The results from the comparison of professional knowledge with the laymen's are profoundly disturbing. In addition, the lack of communication between the scientist and the clinician indicates that changes must be made in education, design, editing and writing of scientific reports. Lack of understanding of statistical language and procedures discourages private investigation and understanding of important research affecting the profession.

333. Calcification Sequence in Developing Enamel and Dentin of the Rat Incisor.—*William C. Rubach and G. Van Huysen, School of Dentistry, Indiana University, Indianapolis.* In the past the formation and calcification of dentin and enamel have been studied by using stained histologic sections from decalcified developing tissues of animal and human jaws. These older methods provided indirect estimations of development, calcification, and maturation of enamel based on the amount of residual matrix material visible after decalcification of the tissues. In the present study, direct determination of the intensity and extent of calcification of enamel and dentin matrix of the developing tooth was made by X-ray micrographic methods. Undecalcified, paraffin-imbedded, 10-μ-thick sections of newborn to 10-day-old hamster jaws were used. X-ray micrographs of the unstained sections were compared and contrasted with alternately cut sections stained with hematoxylin and eosin. Comparisons also were made with histologic sections of decalcified tissues stained with hematoxylin and eosin. Differences in the degree and amount of calcified matrix in the developing tooth enamel and dentin were determined by linear and microdensitometer measurement of the historadiographs. These variations were then easily compared with similar slides of decalcified tissues stained with hematoxylin and eosin. The degree of radio-opacity of the microradiographs shows that the dentinal matrix preceded the enamel matrix in extent and degree of mineralization. Up until the age of 10 days the enamel mineralization closely followed that of the dentin but did not equal it in extent or intensity.

334. (Not presented.)

335. Adrenalectomy Effect on Temperature and Epidermal Mitotic Rhythms in Hamsters.—*A. P. Chaudhry and F. Halberg, Schools of Dentistry and Medicine, University of Minnesota, Minneapolis.* Rectal temperatures were measured on 7 bilaterally adrenalectomized and 7 sham-operated hamsters, every 12 hours during the fourth and fifth postoperative days. Conditions were standardized for light-synchronized periodicity analysis, with light from 06:00 to 18:00. Rectal temperatures of sham-operated animals at 08:00 and 20:00 differed by 1° C. on the second day of sampling but not on the first. The corresponding difference in rectal temperatures of adrenalectomized hamsters was smaller than that of sham-operated controls, and the former, as a group, had consistently a mean rectal temperature that was lower then that of the latter. After detection of this decreased amplitude of temperature rhythm and relative hypothermia following adrenalectomy, samples of ear pinna were cut off for histology, starting at 20:00 on the fifth postoperative day and continuing at 4-hour intervals until 16:00 on the sixth day postoperative. Twenty adrenalectomized and 19 sham-operated hamsters, each provided consecutive 4 hourly samples (serial dependence). From 6 additional subgroups, each consisting of 7–9 sham-operated and 9–12 adrenalectomized animals, one sample only was taken—separate subgroups being studied at 4-hour intervals (serial independence). The rhythm in pinnal mitosis was obliterated in adrenalectomized hamsters, while it stood out clearly in sham-operated animals, irrespective of sampling procedures. The peak-to-trough difference of the sham-operated animals was greater with serially independent sampling than with serial dependence. These studies underline the critical role of the mammalian adrenal cortex in the co-ordination of physiologic function along the 24-hour scale and reveal, in addi-

tion, distinctive effects of gland removal. Adrenalectomy lowers the amplitude of the hamster's temperature rhythm and obliterates its pinnal mitotic rhythm.

336. SERUM IONIZED-CALCIUM METHODS.—*Frances A. Harnach and T. B. Coolidge, Zoller Memorial Dental Clinic, Univeristy of Chicago.* The dye murexide, which combines with calcium with a change in color, has been used by direct addition to saliva and addition to serum ultrafiltrates to measure the calcium-ion concentration. In whole serum an anomalous absorption interferes with the measurement. The effect of the anomalous absorption in serum cannot be obviated by the differential reductants nor by the removal of the heavy metals from the serum. The cause of this interference is still unknown.

337. ZONATION IN CARIES OF THE ENAMEL.—*T. B. Coolidge and Barbara Wallace, Zoller Memorial Dental Clinic, University of Chicago.* With mercury sulfide impregnation of cross-sections exactly located in longitudinal sections, the sheath spaces between prisms and interprismatic substance are seen to be open in the deeper layers of caries, the rods are decalcified in more advanced areas, while the interprismatic material resists decalcification until a very late stage. There is no discontinuity in the impregnation corresponding to the zone of positive birefringence sometimes seen in sections imbibed with organic solution. The sequence of impregnation confirms the previous demonstration that the first two phases of the carious process specifically reverse the last two phases of enamel decalcification: the closing of the sheath spaces and the calcification of the rods.

338. THE EFFECT OF RETRACTION MATERIALS ON THE GINGIVAL SULCUS EPITHELIUM.—*James D. Harrison, School of Dentistry, St. Louis University.* Three dogs were used as the experimental animals. Six retraction materials were used. Each animal was anesthetized with animal pentothal by intravenous injection. The labial or buccal sulcus was retracted with each material for periods of 5, 10, and 30 minutes. The lingual sulcus served as the control. Block specimens including the teeth and soft tissues were taken immediately, at 7, 10, 14, and 21 days. Specimens were fixed, decalcified, and processed for histologic study. All specimens were stained with hematoxylin and eosin. The criteria of injury were stripping, desquamation of epithelium, intracellular hydropic degeneration, hyperemia, inflammation, epithelial proliferation, and necrosis. Microscopic sections were obtained by cutting labio- or buccolingual block sections. The relative degree of injury was recorded as normal, slight, moderate, or severe. Untreated string and string saturated with 1:1000 adrenalin did not cause injury in the 5- and 10-minute periods. Slight injury was found in the 30-minute specimens that healed within 10 days. Strings saturated with 8 per cent adrenalin and 100 per cent alum caused slight injury in the 5- and 10-minute periods and healed in 10 days. The 30-minute specimens healed in 14 days. The zinc chlorides (8 and 40 per cent) caused severe injury in the 5-, 10-, and 30-minute periods and were *not* healed in 21 days.

XXVIII. SYMPOSIUM. MARCH 26, AFTERNOON

RELATIONSHIP BETWEEN RESEARCH, TEACHING, AND PRACTICE IN THE FIELD OF OCCLUSION

(*Note.*—Bridging the gap between research findings and teaching and practice is a central problem in dental education. Because the field of occlusion encompasses every discipline of dentistry, it lends itself well to a discussion of the central problem. In the past, a great deal of confusion has arisen in the minds of students and future practitioners of dentistry because the orthodontist has taught one aspect; the prosthodontist, another; and the periodontist, still another.)

S1. CONTACT PATTERNS USING MINIATURE RADIO TRANSMITTERS.—*Barrie R. D. Gillings and Jacques T. Kohl, Eastman Dental Dispensary, Rochester, New York.* Investigations of occlusal contact patterns have been hampered because existing techniques for recording such patterns may affect the subjects' normal function. In an

effort to conduct such studies without using bulky or uncomfortable intra-oral recording devices, self-contained miniaturized radio transmitter units of the same size and shape as two-unit fixed bridges were constructed for subjects having two or more of their natural teeth missing. A radio signal was produced on tooth contact and could be received on a marine band transistorized radio receiver (1.85–4.20 megacycles) placed up to 6 feet away. For each subject a plastic inlay or onlay was constructed and fixed in position. Two exposed gold contacts were provided on the inlay, with a means for connecting them to the power circuit of the transmitter unit. The wiring was arranged so that when these two gold contacts were joined, a signal was broadcast from the transmiter. A very small gold inlay was placed on the cusp interdigitating with the gold contacts in such a position that the inlay joined the gold contacts only when the teeth were in the particular occlusal contact position being investigated. Positions investigated included the most retruded position and 1 mm. anterior to the most retruded position, with and without premature contact in the contact area. Data on frequency and duration of contact were recorded both direct from the receiver and from 8-hour tape recordings for subjects while chewing and swallowing and while awake and asleep. The method was found to be sensitive and precise.

S2. Craniofacial Characteristics in a Representative Population.—*Egil P. Harvold and Margaret E. Hatton, Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada.* Lateral cephalograms of 4 groups of children aged six, eight, ten, and twelve years were studied to determine (1) changes in craniofacial characteristics at different age levels, the sexes compared, and (2) whether or not any relation which might be found between characteristics acts as a predisposing factor in the development of malocclusion. The four characteristics measured, included (1) cranio-facial index

$$\left(\frac{\text{masion} - \text{pogonion}}{\text{greatest depth of the cranium}} \times 100\right);$$

(2) cranio-occlusal angle (point of intersection of Bolton point and nasion, and the occlusal plane); (3) profile angle (nasion, subnasale, pogonion); and (4) distances between subnasale and supramentale projected at right angles to the occlusal plane. Results showed that the craniofacial index increased with age in both sexes; the cranio-occlusal angle remained very similar at six, eight, and ten years, but at twelve years it was reduced in both males and females. The profile angle was consistently smaller in females at all ages. The range for characteristic 4 was greater in males than in females except at age six. The forward growth of the mandible was greater in females until the age of twelve. Retrognathism was consistently greater in males until this same age. At twelve years, the relation of supramentale to subnasale was almost identical in both sexes. A correlation between profile angle and the relation that the supramentale bears to the subnasale was observed at all ages and particularly at age twelve. This correlation seems to indicate that these two morphologic features act as predisposing factors in the development of malocclusion.

XXIX. DENTAL MATERIALS GROUP

M1. Titratable Acidity, pH, Hardness of Silicates with Different Operators and Conditions.—*Michael G. Buonocore, Tatsuo Suzuki, and Richard H. Roydhouse, Eastman Dental Dispensary, Rochester, New York.* Variations in titratable acidity (TA), pH, and hardness (H) of three popular silicates when mixed by five operators were studied under two conditions, namely, (1) when the powder/liquid ratio was as suggested by the manufacturers and the mix completed in 1 minute (standard mix) and (2) when the operator was given the same quantity of liquid as in 1 and instructed to incorporate powder as needed to produce the kind of mix he would employ clinically (clinical mix). After setting 15 minutes, disks were immersed in 25 cc. of water for 24 hours; then the pH of water was taken and titrated with base to

the phenolphthalein end-point to determine TA. Hardness (Kentron*) was measured after approximately 2 months' water storage. The following conclusions, supported by an analysis of variance, were drawn: Clinical mixes produced higher TA and were softer than standard mixes. S.S.W.† had lowest TA with both types of mixes. With standard mix, Caulk‡ and Vivadent§ showed the same TA, but with clinical mix the Vivadent had significantly higher TA than Caulk. There was considerable difference in TA with the same material for different operators under both conditions, although the variation was three times as great with clinical as with standard conditions. Hardness appeared to be inversely related to TA. Vivadent disks, air-dried 10 minutes after 2 months in water, showed marked surface whitening, some loss of polish, and stained heavily in dye. Caulk showed some opaquing and both S.S.W. and Caulk stained moderately with dye. Assuming that TA is related to pulp injury and H is a measure of in vivo durability, better results will be obtained by mixing according to directions.

M2. Silico-Phosphate Cements as Cementing Media.—*George C. Paffenbarger, American Dental Association Research Division, National Bureau of Standards, Washington, D.C., and John N. Anderson, University of St. Andrews Dental School, Dundee, Scotland.* Silico-phosphate cements are essentially silicate cements containing sizable quantities of zinc oxide in the powder. Usually this zinc oxide is fused into the powder, but in at least one instance the powder is a mixture of silicate and zinc phosphate cement powders. Some of the silico-phosphate cements are recommended for both cementing and filling operations. This report deals with their use as cementing media. Seven products of American and European origin were subjected to the tests outlined in American Dental Association Specification No. 8 for dental zinc phosphate cement. The test data for consistency ran from 0.55 to 1.35 gm. of powder (in 0.5 ml. of liquid) to produce a disk 30 mm. in diameter; for time of setting, from 8 minutes to over 1 hour; for film thickness, from 0.36 to 0.053 mm.; for 24-hour compressive strength, 14,600–18,900 psi; and for opacity, from 100 per cent to the 35–55 per cent range. On the basis of the foregoing data, one wonders whether the silico-phosphate cements are more suitable cementing media than zinc phosphate cements. Some rigorous clinical testing is needed for a decision.

M3. Influences of Specific Surface on Some Properties of Silicate Cement.—*Gerald T. Charbeneau, University of Michigan, Ann Arbor.* The powder-liquid ratio is generally known to have a great influence on the physical properties of silicate cement. The particle size and particle-size distribution of silicate powders has received little attention in the dental literature. A preliminary investigation was thus undertaken, using known relative specific surfaces of a single batch of silicate powder, to determine the influence of this variable on the physical properties. Silicate powders with specific surface of 2,160 cm^2/gm, 2,540 cm^2/gm, 3,030 cm^2/gm, and 3,410 cm^2/gm were used. The standard consistencies determined were found to vary considerably. Setting time was 3 minutes for the powder of greatest specific surface and increased as the surface decreased. Compressive strength was found to increase with the specific surface, even though the powder-liquid ratio was steadily decreased, in order to form the standard consistencies. The Knoop hardness of the silicate matrix formed with these powder samples varies between 67 and 84 KHN at standard consistencies. The powder-liquid ratio of the 2,160 cm^2/gm silicate sample was varied from 1.5 to 1.0 gm/0.4 ml. The hardness of the matrix decreased from 84 to 45 KHN. Knoop hardness of the silicate particles themselves averaged 547 with a 100-gm. load. Further studies are under way to determine the full significance of specific surfaces on the physical properties.

* Christian Becker Co., Clifton, New Jersey.

† S. S. White Dental Manufacturing Co., Philadelphia, Pennsylvania.

‡ L. D. Caulk Company, Milford, Delaware.

§ Vivadent Corporation, Boston, Massachusetts.

M4. Structure and Properties of Gold Foil and Mat Gold.—*Jean T. Hodson, University of Washington School of Dentistry, Seattle, Washington.* A previous study on hardness led to more detailed examination of the underlying structures of gold foil and mat gold. The purpose of this investigation was to study the porosity of condensed gold foil and mat gold. Forty specimens were prepared in Lucite* and measured for hardness. Samples of these were etched, and some were sectioned for examination under the microscope. Important phases were photographed for comparison and illustration. Structures observed in the condensed specimens included laminations of dense gold, voids, uncondensed gold, and condenser imprints. The physical properties revealed by these structures included strain hardening, stress relief, solubility, cohesion, density, and plasticity. Evidence of high stain in condensed gold foil was observed by the formation of crystal grains in the polished surface layer. With twelve slip systems available to operate simultaneously in the randomly oriented gold foil and mat gold, it was possible for the gold to be sufficiently hardened during placing and initial compaction to resist subsequent forces. The result was loosely or moderately compacted gold sandwiched between thin, dense layers. These were observed in cross-sections of the condensed specimens. The findings of the investigation lead to a better understanding of the mechanics and physical properties involved in the condensation of gold foil and mat gold.

M5. Compressive-Strength Machine for Impression Materials.—*James Cresson, L. D. Caulk Company, Milford, Delaware.* A description was presented of a compressive-strength testing machine for elastic impression materials. This was described to apply load at the rate of 18,000 gm. per minute, as required by the tentative American Dental Association Specification for hydrocolloid impression material-alginate type. It consists essentially of a shot-loading mechanism and a receiver attached to a lever on which is also a piston which applies the load to the test cylinder. The loading mechanism consists of a hopper of shot connected to an inclined auger, which is turned by a variable-speed motor. Shot raised up the incline spills from the auger tube into the receiver on the counterbalanced lever arm. The rate of shot delivery is controlled by adjustment of the motor speed. The counterbalanced receiver makes it possible to start the test with zero load on the test specimen. The piston and shot receiver make it possible to start the test with zero load on the test specimen. The piston and shot receiver are so located on the lever arm that a 5:1 load ratio exists, thus necessitating a smaller amount of shot being delivered. This makes it possible to operate the auger at a moderate and feasible speed and to have better control over the rate of loading.

M6. Space Formation around Wax Patterns during the Normal Setting and Hygroscopic Expansion of Investments.—*George Mumford, Indiana University School of Dentistry, Indianapolis.* This study was undertaken to determine whether spaces form around a wax pattern during the setting reaction of gypsum investment materials and, if so, what part they play in the final dimension of the mold. Wax patterns were poured in a stainless-steel MOD die and held under pressure until cooled. They were then invested, using a variety of techniques involving both normal setting and hygroscopic expansion. Patterns were sprued on either the occlusal or the proximal surface. After the investment had set, it was removed from the ring and the excess pared away until approximately 4 mm. remained surrounding the pattern. This was dried in a desiccator over calcium chloride and then impregnated with a low-viscosity, epoxy-type laminating resin. When the resin had polymerized, the block was sectioned and the wax removed with hot water. The sections were then polished by means of a range of emery polishing papers. Sections were cut in both a mesiodistal and a buccolingual direction. The thickness of the resin film which flowed into spaces around the pattern was measured. Spaces formed in every technique studied, and, although irregular in form, they were symmetrically related to the sprue. These spaces

* Du Pont de Nemours & Co., Wilmington, Delaware.

occurred on the outer or non-fitting surface of the pattern and also at the gingival floor and the buccal and lingual walls of the occlusal surface and the proximal box. The spaces formed on the fitting surface could affect the dimension of the casting, and in one typical area—the gingival floor—they varied from a maximum of 0.12 mm. in a hygroscopic technique to 0.01 mm. in the normal setting expansion of an investment utilizing the thermal expansion technique.

M7. The Effect of Accelerators and Retarders on the Setting and Crystal Structure of Plaster.—*Y. G. Sunoo, D. C. Smith, and E. W. Skinner, Northwestern University Dental School, Evanston, Illinois.* The effect of certain accelerators and retarders on the setting of the α- and β-hemihydrates of calcium sulfate and on the crystallization of the resulting gypsum was studied. The setting properties were studied by determining setting time, linear setting expansion, compressive strength, and degree of hydration by exotherm. Crystal habit and size were studied microscopically for dilute suspensions and for the set mass. The various accelerators and retarders were observed to produce marked changes in crystal habit, size, and rate of nucleation, which were dependent on the concentration of the particular agent. Most accelerators—for example, potassium sulfate—produced long acicular crystals and an increased rate of nucleation, whereas retarders, such as borax, produced smaller, squat crystals and a slower nucleation. Sodium chloride caused a gradual change from acicular toward a more platelike habit with increasing concentration. The results of the study provided an explanation for the effects of the various modes of action of accelerators and retarders on the setting properties of plaster.

M8. Factor Influencing the Effective Setting Expansion of Casting Investments.—*A. Bruce Ady and David B. Mahler, University of Oregon Dental School, Eugene.* In dental casting procedures, consideration of the setting expansion of casting investments is necessary for proper compensation of gold and wax shrinkages. Since it has been shown that investment expansion is influenced by restrictive forces, there has been some question concerning the validity of traditional expansion measurements taken of an investment mass setting in a trough, in predicting the actual or effective expansion which occurs relative to the wax pattern in the invested ring. Consequently, it was the purpose of this investigation to determine the effective setting expansion of investments in casting rings, with particular emphasis on the effect of manipulative variables and the effect of the asbestos liner. Measurements were made to marks in a single-surface wax pattern and to corresponding marks in the set investment. The accuracy of measurement was established at 0.10 per cent. From these tests, it was determined that in techniques in which the expansion capacity of the investment was restricted by the asbestos liner, the nature of the placement of the liner had a pronounced effect on the effective expansion, whereas variations in water/powder ratios and degree of spatulation did not. In techniques where the expansion capacity was not restricted by the asbestos liner, manipulative variables appreciably influenced effective expansion, whereas the nature of placement of the liner did not.

M9. Permeability of Investment at the Casting Temperature.—*John S. Shell* and Edward R. Dootz, Ransom & Randolph Co., Toledo, Ohio.* An apparatus has been developed which is capable of measuring the flow of gas at a controlled pressure through a predetermined thickness of investment at the casting temperature. The gas permeability of investments which have been heated and then cooled to room temperature are of minor significance because of the effect of the shrinkage of the investment during cooling. This may introduce minute cracks in the investment, which would increase the permeability, or the shrinkage might increase the density of the investment, causing a reduction in the permeability. Tests at the casting temperature are much more significant. The tests at the casting temperature indicated a considerable variation in permeability, depending on the investing technique used, with vacuum

* Present address: J. F. Jelenko Co., New York City.

investing showing a higher permeability. Wax burned out in the investment also increased the permeability. The permeability of "green" investment was so small that no reading was obtained on the flowrator. The permeability readings start at 15–20 minutes after the investment is placed in a furnace at 850° F., and the maximum permeability is reached rapidly thereafter.

M10. The Retardation of Gypsum Plaster by Calcium Tartrate.—*Joan A. Donnison, Maxwell P. Chong, and Alan R. Docking, Commonwealth Bureau of Dental Standards, Melbourne, Australia.* It was found that the effect on the setting of gypsum plaster by rochelle salt or similar tartrates was entirely different when a small amount of calcium oxide was present in the plaster. This was discovered when the setting rate of a mixture of equal proportions of such a plaster and a hydrocal containing rochelle salt was examined. An investigation into the explanation for this phenomenon was carried out by examining the rates of setting and evolution of heat and the change in the crystal habit. It was found that the effect could be reproduced by (1) mixing a lime-free plaster with an alkaline solution of calcium tartrate, or (2) mixing the hydrocal containing rochelle salt with an alkaline solution. Microscopic examination showed that the crystal habit was markedly changed to a more tabular form.

M11. (Not presented.)

M12. Mercury Micromeasurement for Determining Accuracy of Inlay Castings.—*E. A. Sahs and J. H. Wick, College of Dentistry, State University of Iowa, Iowa City.* This paper is a report of work done in continuation of previous efforts as presented at the 1960 meeting of the Dental Materials Group in Chicago. A comparison was made of various techniques employed to determine the volume of space which exists between the cavity walls and the casting, as an indication of fit. The first phase of this project was the standardization of wax-pattern procedure. Wax patterns of cavities prepared in metal dies were taken at room temperature. On the basis of the manufacturers' data, the second phase of the project was concerned with the investing, burn-out, and casting. Eleven different investments were categorized, and base lines were established for controls. The third phase dealt with determining the volume of space between the casting and the walls of the cavity. After trying several materials, such as light mineral oil, glycerin, dibutyl phthalate, zinc oxide and eugenol, and mercury, it was found that mercury was the most desirable because its high specific gravity made small differences in castings easily measurable. The method used to determine volume of space was the straight displacement method, and from the microweight and specific gravity the volume was computed.

M13. (Not presented.)

M14. Test Programs for the Statistical Evaluation of Dental Materials.—*W. J. Youden, National Bureau of Standards, Washington, D.C.* Dental materials are, to an exceptional degree, under continuous development and trial. These materials must meet exacting requirements for their composition and properties. Laboratory tests are used to screen materials that are finally submitted to clinical tests for workability and durability. Clinical testing presents unusual difficulties in the control of the test area and in the objective appraisal of performance. For this reason, it is desirable to make comparative tests—i.e., new materials matched against materials of known performance. The effectiveness of comparative testing may be increased by an appropriate schedule of comparisons, especially if only two materials can be contrasted under the same conditions. In brief, it is more efficient to test all possible pairings (or a carefully chosen subset) than it is to carry along a "control" in each comparison. Sometimes the appraisal of performance is limited to the indication of which one of two materials is the better. A statistical procedure exists for establishing an over-all ranking of many materials given such preference choices for pairs alone. There seems to be an opportunity here to increase the number of individuals participating in conducting such comparisons and thus achieve a broader and sounder basis for drawing conclusions.

M15. Effect of Casting Temperature on the Properties of Chrome-Cobalt Alloy.—*Duane F. Taylor, National Bureau of Standards, Washington, D.C., and Edmund S. Olsen, Jr., Central Dental Laboratory, Walter Reed Army Medical Center, Washington, D.C.* The tensile strength, yield strength, modulus of elasticity and per cent elongation were determined for a series of Ticonium* castings. The casting temperature was controlled by means of a two-color optical pyrometer,† and castings were made at 25° F. intervals across the entire clinically useful range. Castings were made from new metal, 50 per cent new–50 per cent scrap metal, and 100 per cent scrap metal. Plots of the values for individual properties versus casting temperature were commonly found to exhibit maxima. The optimum temperatures were found to vary with the type of metal used and, to a lesser extent, with the quantity of metal cast. The average properties at the optimum temperature were significantly better than those for specimens cast at less closely controlled temperatures.

M16. The Effect of Casting Temperature on the Dimensional Accuracy of Cobalt-Chromium Dental Castings.—*Edmund S. Olsen, Jr., Walter Reed Army Medical Center, Washington, D.C., and Duane F. Taylor, National Bureau of Standards, Washington, D.C.* The objective of this investigation was to determine the effect of control of casting temperature upon the dimensional accuracy of cobalt-chromium dental castings. Temperature control was made possible through the use of an automatic two-color pyrometer,† previously described. It was installed on a Williams‡ induction casting machine, and used under actual operating conditions. The installation included a device for automatic release of the casting-machine carriage when the preset temperature was reached and for control of the timing of the spin cycle. A die was fabricated which resembled a partially edentulous maxilla with four tapered abutments located in positions corresponding to the cuspids and second molars. Rest seats were prepared in those abutments, and sharply defined indentations were made on the crest of the residual ridges approximately 5 mm. distal to the anterior abutments and 5 mm. mesial to the posterior abutments.§ The distance between indentations was measured to the nearest 0.001 inch with a measuring microscope. An average of four separate measurements of each dimension was made to insure reading accuracy. Tests verified the reproducibility of the reference marks in refractory casts as well as in cobalt-chromium castings. Duplicating, wax-up, and investing methods and materials were critically controlled to minimize variables other than those directly related to casting temperatures. Findings indicated that it is possible to determine an optimum range of casting temperatures for dimensional accuracy of cobalt-chromium dental castings of various sizes and that these temperatures can also be related to the physical properties of such castings.

M17. Effects of Different Investments on the Sulfide Contamination of the Imbedded Metals in Casting Procedure.—*Kamal Asgar, University of Michigan, Ann Arbor.* The use of wrought wire as partial denture clasps instead of cast clasps is an accepted procedure for the construction of removable partial dentures. Some prefer to solder the wires after the casting is completed, whereas others have eliminated the soldering operation by casting directly to the imbedded wires in the investment. This technique is used frequently with Ticonium* base metal-alloy cast to PGP|| precious-metal alloy wire. This wire, however, may also be used in connection with dental gold-casting alloys. Occasionally, the imbedded wire is damaged during casting, which may cause it to break easily either during the adjustment of the clasp or in service. In this study photomicrographs of PGP wires under different casting

* Ticonium Division, Albany, New York.

† Shawmeter.

‡ The Williams Gold Refining Co., Inc.

§ These reference marks were made at the National Bureau of Standards with a Knoop Indenter.

|| Platinum-Gold-Palladium.

conditions were studied. The photomicrographs revealed that in casting to the imbedded metals the choice of the investment is important. The investment must have some protective additives to prevent the PGP wire from sulfidizing. It was also shown that the Ticonium investment, by having such an additive present, does not damage the PGP wire when handled properly, regardless of the type of the alloy cast into the mold. The regular investments for dental gold alloys usually damage the surface of the PGP wires when the wires are imbedded in the investment, since they do not contain such additives.

M18. Dimensional Changes during Duplication of Stone Casts.—*D. H. Anthony, R. G. Craig, and F. A. Peyton, University of Michigan, Ann Arbor.* The importance of dimensional changes during duplication indicated the need for horizontal and vertical dimensional measurements on stone and duplicate refractory casts. Agar and plastic-type duplicating compounds and gypsum, silicate, and phosphate-bonded investments were used with various duplicating procedures. An average-size stone cast containing no undercuts and four cylindrical posts in the second molar and cuspid regions was used as the master cast. All possible post-to-post distances were measured on the stone and duplicate casts, and an average per cent horizontal change was calculated. Hemispherical indentations in the top of the posts and the palatal area permitted the use of a comparator to determine the vertical dimensional changes. The investment casts of the gypsum type and one phosphate-bonded type were larger than the masterstone cast. The investment casts of the silicate and one phosphate-bonded type were essentially the same size as the master cast. In general, the gypsum-bonded investment casts were smaller than normal when (1) the agar gels were stiffer, (2) the sol pouring temperature was higher, (3) the cast temperature was higher, and (4) the investment was immersed in water during the setting reaction. Little effect on dimensions was observed as a result of flask-type or aging of the agar, using a non-undercut cast. Gypsum and phosphate investments which expand during setting showed somewhat larger per cent vertical than horizontal changes. Although the silicate-bonded investment casts showed no horizontal changes, vertical shrinkages of more than 1 per cent were observed.

M19. The Accuracy of Large Investment Casts.—*R. H. Roydhouse and E. W. Skinner, Eastman Dental Dispensary, Rochester, New York, and Northwestern University Dental School, Chicago, Illinois.* Previous experimental data (unpublished) showed that the dimensional changes due to the duplicating process when investment models are made are not directly related to the measurement of the setting expansion of investment in asbestos-lined casting rings. The effects of the position of the master cast in the duplicating flask and the thickness of the base of the cast on the dimensions of the investment model were investigated. A metal die resembling a transverse section of a maxillary cast was duplicated by means of a hydrocolloid in a flask, such as those used in many dental laboratories. The investment models were measured in the horizontal and vertical directions, and the measurements were compared by the analysis of variance. From the table showing the changes in dimension under six different circumstances related to the thickness of the base and the orientation of the master cast in the duplicating flask, it was concluded that (1) the base of the investment cast should not be less than $\frac{1}{2}$ inch in thickness in any part; and (2) the design of the duplicating flask should be such that the critical parts of the master cast are surrounded on all sides by hydrocolloid. The setting expansion of the investment distorts the cast less with a $\frac{1}{2}$-inch-thick base, and the free expansion of the investment occurs in a direction that does not affect the accuracy of the investment cast and thus of the final casting also. In most duplicating flasks the free vertical expansion of the investment increases the length of the teeth more than the horizontal dimensions of the cast, and thus the incisal and occlusal rests of castings sometimes fail to seat on the master cast or in the mouth.

M20. THERMOGENICS IN CAVITY PREPARATION USING THE AIR-TURBINE HANDPIECE.—*A. W. Aplin, F. M. Sorenson, and K. R. Cantwell, University of Oregon Dental School, Eugene.* The cutting of the internal detail of cavity preparation with the air-turbine handpiece requires unimpaired visualization of the tooth. The use of an air coolant during this procedure satisfies this requirement. The purpose of this study was to examine the thermal production associated with the use of an air coolant in a two-phase procedure (rough-out and finish) of cavity procedure. The in vitro detection of temperature rise was accomplished by means of a thermocouple placed at a specified distance from the terminal cutting activity. Under conditions of standardized load, varying air pressure to the handpiece, and burs of different types, class V cavity preparations were cut in extracted bicuspid teeth while the temperature variation was recorded. The results of this investigation indicated that significantly decreased temperature increases are produced when using (1) the #170 over the #35 carbide bur; (2) the stall-out pressure over full air pressure to the handpiece in the finishing phase; and (3) the stall-out pressure during the finishing phase over full air pressure to the handpiece during the roughing-out phase of the class V preparation using air as a coolant.

M21. PRELIMINARY STUDIES ON THERMAL PRODUCTION BY SOME COMMONLY USED POLISHING PROCEDURES IN DENTISTRY.—*F. M. Sorenson, A. W. Aplin, and K. R. Cantwell, University of Oregon Dental School, Eugene.* Because the finishing and polishing of dental restorations unquestionably enhances the quality of these restorations in several ways, most dental practitioners routinely include such procedures as a part of their regular operative technique. That considerable heat is generated by at least some polishing methods used in dentistry is quickly realized by anyone who attempts to polish rapidly a restoration such as a gold crown while holding it in his fingers. It is reasonable to expect that the polishing of restorations which have already been placed in a tooth may contribute significantly to the total thermal experience of that tooth and should not, therefore, be overlooked in our attempts to discern important aspects of heat production in clinical practice. Data obtained from carefully controlled in vitro studies in our laboratory suggested that the heat which is produced during application of certain widely used polishing methods may prove to be important in future considerations of the physiologic and pathologic responses of teeth to thermal stimuli arising from clinical operative procedures.

M22. CRACKS IN TOOTH STRUCTURE ASSOCIATED WITH ROTARY CUTTING INSTRUMENTS.—*Z. Kasloff, Faculty of Dentistry, University of Manitoba, Winnipeg, Manitoba, Canada.* Previous studies of the effect of rotary cutting instruments on tooth structure have concerned themselves largely with pulpal reactions, surface characteristics of cavity walls, and margins and cutting efficiency. This investigation was undertaken to determine what effect various rotary cutting devices had on crack production in tooth structure and to develop an applied visual inspection technique for demonstrating this phenomenon. Two hundred lower anterior human teeth which had been extracted in the oral surgery clinic were stored in water and used in this study. The technique of penetration with a fluorescent dye and visual inspection under ultraviolet light successfully used in engineering and industry was developed and applied in this study to demonstrate pre-existing or newly induced cracks in the specimens selected for this investigation. Conventional and high-speed rotational cutting instruments were used. In addition, an ultrasonic instrument was included in the study because of its recent popularity and interest. These teeth were treated with the fluorescent penetrant and photographed before and after instrumentation under ultraviolet light. The films were mounted on a slide and evaluated with respect to crack formation. Every type of instrument equipped with different cutting tips and at various speeds produced cracks in teeth, but not at all times. Some instruments produced the effect with greater frequency than others. The type of cutting tip used with the rotating instrument appeared to influence the frequency and severity of cracking produced with regularity.

M23. Physical Properties of Root-Canal Files and Reamers.—*R. G. Craig and F. A. Peyton, University of Michigan, Ann Arbor.* The physical sizes of conventional endodontic instruments have been reported. In this study, the stiffness in bending, the permanent set after angular deflection, and the resistance to breakage by 90° bending and to 180° twisting were related to the size and shape of the root-canal instruments. Conventional files (Nos. 1–6) and reamers (Nos. 1–12), as well as new standardized instruments (Nos. 10–100), were included. A limited comparison was made between carbon and stainless-steel instruments of sizes 10–100. The bending moment versus angular deflection was determined between 0° and 90° deflection over a span 3–14 mm. from the tip of the instrument. These curves indicated the stiffness and yield point in bending. The permanent set after angular deflection of 30° and 90° was also measured. The resistance to breakage by bending or twisting was measured by determining the number of 90° bends or 180° twists withstood before fracture occurred. The stiffness curves for comparable-size files and reamers were quite similar. In general, the stiffness of the conventional reamers Nos. 1 and 2 was greater than for the comparable files, the stiffness of Nos. 3 and 4 files and reamers were nearly identical, while Nos. 5 and 6 reamers were less stiff than the comparable files. The new standardized reamers, however, were generally stiffer than comparable files. Other than for a few exceptions, which may be explained as being related to the shape of the instrument, the larger the instrument, the less the resistance to permanent set and breakage by bending or twisting.

M24. The Tin-Mercury Phase in Dental Amalgam.—*Carl W. Fairhurst and Gunnar Ryge, Marquette University, Milwaukee, Wisconsin.* Crystals of tin-mercury were obtained by placing alloys of the silver-tin system in contact with mercury. The flat hexagonal crystals obtained in this manner were used for X-ray diffraction studies. Mixtures of tin and mercury from 5 to 70 weight per cent mercury were also studied by X-ray diffraction. To establish the relationship between data for grown crystals and for tin-mercury mixtures with X-ray diffraction data from dental amalgam, a number of dental alloys were mixed with mercury and studied by the use of X-ray diffraction. Hexagonal symmetry elements and approximate unit-cell dimensions were obtained from the grown crystals. These data were used to index the powder patterns of tin-mercury mixtures of 35–70 weight per cent mercury. The unit-cell parameters of the hexagonal tin-mercury phase were found to be $a_0 = 3.202 \pm 0.002 c/a = 0.93$. The X-ray intensity distribution was found to be that of a solid solution. Additional work is now being done on the low mercury-content alloys to determine parameter variations as a function of composition.

M25. Effect of Alloy Annealing Time and Temperature on the Physical Properties of Amalgam.—*E. W. Skinner, L. C. Won, and G. T. Mizera, Northwestern University Dental School, Evanston, Illinois.* Five typical commercial amalgam alloys were heat-treated at 37°, 50°, 100°, and 150° C. for times varying from 1 hour to 24 weeks. The compressive strength and dimensional changes of the amalgams prepared with these alloys under standard conditions were then determined. The expansion during setting was reduced as the time and temperature of alloy annealing was increased. All amalgams shrank when the alloy was annealed at 150° C. for 1 hour. Saturation temperature and time periods were found beyond which no further change in dimension occurred with alloy annealing. Although overannealing tended to reduce the compressive strength of the amalgam, the effect was not of consequence.

M26. Early Flow Tests on Amalgam.—*W. Dean Kimmel, Milford, Delaware.* Data obtained from a modified flow test in which a load of 1,500 psi was supplied on a 4×8–mm. amalgam specimen at 10 or 15 minutes from the start of amalgamation could not be generally correlated with a clinician's opinion of the setting time of an amalgam, but there was considerable difference in the resistance to the early flow of the various alloys tested. One group of alloys had comparatively little flow during the

entire 2-hour loading period used in the test; another group had a high initial flow during the first 10 minutes after the load was applied but very little flow after this time; the third group had considerable flow throughout the test. This is one of the few tests made on amalgam which showed a wide difference in performance of alloys, and a better understanding of its meaning may make it valuable as a specification test.

M27. Effect of Hg/Alloy Ratio on the Transverse Strength of Amalgam.—*David B. Mahler and John C. Mitchem, University of Oregon Dental School, Eugene.* It has recently been determined that variations in the Hg/alloy ratio do not affect the compressive strength of dental amalgam. Since a large number of structural failures of amalgam in clinical practice are fractures at the cavo-surface margin, a transverse strength test was considered to be more predictive of this type of failure. Consequently, the purpose of this investigation was to determine the effect of the Hg/alloy ratio on the transverse strength of dental amalgam. Samples approximating the bulk of an amalgam restoration were condensed into a rectangular mold measuring $1 \times 4 \times 12$ mm. A three-point loading system was employed, and the transverse strength determined for a fine-cut alloy at Hg/alloy ratios of 1:1, 1:1.4, 1:2, and 1:4. No differences in transverse strength were found with these ratios, provided that the same amount of mercury was present in the precondensation mix. In addition, the transverse strength of the dental amalgam studied was determined to be of the order of 17,000 psi, which is about twice the tensile strength and about one-third the compressive strength for this alloy.

M28. Some Tensile Properties of Dental Amalgam.—*Mario S. Rodriguez, Georgetown University, Washington, D.C., and George Dickson and Philip L. Oglesby, National Bureau of Standards, Washington, D.C.* The tensile properties of four brands of dental amalgam were investigated. Amalgam specimens were made in a split dumbbell-shaped mold having a straight portion of 0.01^2 inch cross-sectional area and 0.30 in length. The amalgam was triturated according to the manufacturer's directions with an amalgamator.* The amalgam was then condensed in the mold with a special plugger, using 1,400 ± 100 psi condensation pressure. The tensile properties were determined on a testing machine† using a crosshead speed of 0.003 in/min. Optical-strain gauges were attached directly to the specimen to measure the amount of deformation under load. All specimen preparation, storage, and testing were done in a controlled-temperature room maintained at 23° ± 1° C. and 50 ± 4 per cent relative humidity. The average results obtained and their standard deviation (S.D.) from at least five specimens on a 7-day-old amalgam are given in the table.

Alloy Brand	Chord Moduli of Elasticity in Tension 10^6 Psi from Stress of 1,000 Psi to				Elongation 0.50 In. Gauge Length		Tensile Strength	
	3,000 Psi	S.D.	5,000 Psi	S.D.	Per Cent	S.D.	Psi	S.D.
A*.....	3.3	0.30	2.2	0.13	0.50	0.12	7000	720
B*.....	3.6	0.60	2.6	0.46	0.50	0.16	7600	970
C†.....	3.9	0.55	2.7	0.24	0.45	0.12	8400	760
D*.....	4.1	0.17	2.8	0.12	0.30	0.09	8100	630

* A.D.A. specification type alloy.

† High tin alloy.

M29. Effect of Amalgam Condensation on Compressive Strength.—*G. T. Mizera and E. W. Skinner, Northwestern University Dental School, Evanston, Illinois.* Many investigators have studied the effect of various variables on the condensation

* Crescent Wig-L-Bug. † Instron-Tuckerman.

of amalgam by condensing the triturated mass at one time under a specific static load. This method of condensation using static loads of 32, 128, and 148 pounds, respectively, has been compared with the compressive strengths obtained under similar conditions with controlled hand condensation. It was found that, for mercury/alloy ratios below 1.30, higher compressive strengths were obtained at the end of 1 hour and 7 days with hand condensation than with machine condensation, even though mercury content of the former was higher than in the machine-condensed specimens. With greater ratios, the 1-hour strengths of the specimens condensed under the 128-pound load corresponded quite closely to those obtained by hand condensation, whereas after 7 days the strengths of the hand-condensed specimens more closely corresponded to the strengths obtained with the specimens condensed under the 32-pound load.

M30. Properties of Dental Amalgam Made from Spherical Particles.—*Neil C. Demaree, Georgetown University, Washington, D.C., and Duane F. Taylor, National Bureau of Standards, Washington, D.C.* A study was made of the effect of particle size on the physical properties of dental amalgam. An experimental alloy was prepared and atomized into powdered spherical form. By means of sieving and hydraulic elutriation, the alloy was separated into fractions of eight size ranges, the smallest size range containing particles having a diameter of 4 μ and less and the largest size range containing particles having a diameter of 105–150 μ. Physical properties were determined on dental amalgam using individual size ranges, combination of size ranges, and three commercial-brand alloys as controls. The physical properties investigated by standard methods were compressive strength, dimensional change on setting, flow, and residual mercury content. Particle sizes of 15–50 μ in diameter gave the most desirable physical properties for dental amalgam and compared most favorably with those obtained for the controls. As each extreme of the size ranges was approached, the crushing strength decreased, the per cent of flow increased, and a greater amount of setting change took place. The physical properties of the largest size ranges were more extreme than those of the smallest size ranges.

M31. Bond Strength of Repaired Amalgam.—*Louis G. Terkla, John C. Mitchem, and David B. Mahler, University of Oregon Dental School, Eugene.* When an amalgam restoration fractures, either immediately after placement or after some time in service, an established clinical practice has been to condense freshly mixed amalgam against the remaining portion of the restoration. This practice has received little quantitative evaluation. As a consequence, it was the purpose of this investigation to determine the strength of the bond between the new and old amalgam and to determine how this strength compared to the strength of the original amalgam. A transverse strength test was selected as the method of evaluation. A sample measuring $1 \times 4 \times 12$ mm. was selected to approximate the size of an amalgam restoration. A fine-cut alloy was used with a 1:1 Hg/alloy ratio. Condensation was effected by a small-diameter condenser, so that free mercury was always visible after condensing each increment. The sample was fractured in half in a three-point transverse strength test, and freshly mixed amalgam was condensed against one of the fractured halves. The transverse strength was again determined for the repaired amalgam. Preliminary tests showed that the strength of the junction between the new and old amalgam was approximately half that for the original amalgam.

M32. Amalgam Properties When Mixed by Pressure Only.—*Toshiro Nakamura, Theodore E. Fischer, Hiroyoshi Habu, and Kasuo Nagai, Nihon University School of Dentistry, Tokyo, Japan.* The physical properties of a fine-grain amalgam were compared when amalgam was mixed by means of pressure alone and when mixed with a very popular mechanical trituration method. The crushing strength, mercury-alloy ratios, corrosion tendency, and expansion and contraction of a fine-grain amalgam were checked after mixing amalgam by pressing the mercury through the alloy

powder. The mixes were produced with various pressures (ranging from 1,070 to 5,600 lb/in^2) and at various time intervals. The mercury was placed in a close-ended cylinder, and then the powder was placed on top of the mercury. Pressure was applied to the ingredients through a piston placed on top of the alloy powder, and a controlled lever action produced the pressure. The mercury and powder were weighed in 8 to 5 ratios. After application of pressure the expressed mercury was again weighed to determine the final mercury-alloy ratio. After regulated condensation, the samples were subjected to tests of their physical properties. The results showed that amalgam can be mixed instantaneously with pressure alone.

M33. Some Rheological Properties of Dental Amalgam.—*Philip L. Oglesby, George Dickson, National Bureau of Standards, Washington, D.C., and Mario S. Rodriguez, Georgetown University, Washington, D.C.* An investigation of the mechanical properties of amalgam was made by means of rheological stress-strain-time experiments, from which the creep and creep-relaxation curves were obtained. The creep experiment was performed by placing a dumbbell-shaped amalgam specimen approximately 1 week old under a constant tensile stress and observing the strain at the instant of stress application and over a period of time after the stress was removed from the specimen. From these data, strain-time curves were plotted for various stress levels with different specimens of amalgam made from two commercial bands of alloy. From these curves, the tensile moduli and the coefficients of viscosity for the various stress levels were determined. For brand A, the instantaneous modulus of elasticity E_1 and retarded elastic modulus E_2 were found to be approximately the same over the stress level range 970–4800 psi having the values $E_1 = 5.1 \pm 0.3 \times 10^6$ psi and $E_2 = 5.0 \pm 0.5 \times 10^6$ psi. However, the coefficient of viscosity n_3 decreased with increasing stress. The values of n_3 for brand A were found to be 22.0, 5.3, 2.2, 1.2, 1.6, 1.0×10^9 psi/sec at the respective stress levels 970, 1,900, 2,900, 3,550, 3,860, and 4,800 psi. Strain-time curves were run at only one stress level, 3,550 psi, for brand B. At this stress level, the moduli E_1 and E_2 were found to be 4.8×10^6 psi and 2.8×10^9 psi, and the coefficient of viscosity n_3 was found to be 4.2×10^9 psi/sec. The values reported are based on preliminary results of work now in progress.

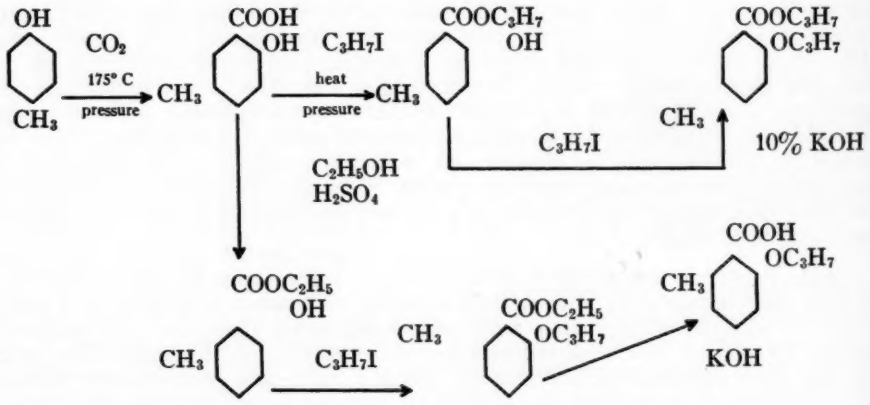
M34. Polishing of Amalgam.—*Joseph Rubinstein and Maury Massler, University of Illinois College of Dentistry, Urbana, Illinois.* Amalgam disks were prepared in acrylic forms and polished with various agents 1 or 7 days after production. The surfaces were then compared for degree of polish, reduction of surface level, and effects on marginal adaptation. Polished and unpolished surfaces were exposed to the action of sulfides, acids, alkalis, and galvanic current. Polishing with flour of pumice followed by whiting produced the highest luster with least loss of surface material and least disturbance of marginal continuity. Polishing 7 days after the initial set produced a much smoother surface than polishing after 24 hours. Sulfides and acids produced severe tarnish on unpolished surfaces but also tarnished polished surfaces more slowly. Alkalis retarded tarnish and increased polish. When amalgam disks were made the positive poles in galvanic cells containing sulfide as the electrolyte, the surface tarnished as when immersed in acids. When the disks were connected to the negative pole, tarnish was retarded in a manner similar to that seen when the disks were placed in alkalis.

M35. Progress Report on Clinical Study of Amalgam Fillings.—*Charles J. Wilson and Gunnar Ryge, Marquette University, Milwaukee, Wisconsin.* In a clinical study of amalgam fillings made from four experimental alloys and two commercial alloys, 500 fillings were observed over periods of 6, 12, and 18 months. The experimental alloys included zinc and non-zinc alloys of large- and small-particle thickness, the properties of which were also studied by laboratory methods. Records were kept of several manipulative variables, such as cavity preparation, condensation technique, contamination, matrix stability, and finishing procedure. The incidence and severity

of tarnish and corrosion, marginal breakdown, and fracture were noted at semiannual recalls, and work is presently in progress to establish the statistical significance of these findings as correlated with particle size, zinc content, and the various manipulative factors. Preliminary analysis indicated that the effect of the manipulative variables was more pronounced than differences due to zinc content or particle size.

M36. Investigation of Hygroscopic Investment (Control Water Added) for Casting Multiunit Bridges.—*P. W. Herrick, College of Dentistry, State University of Iowa, Iowa City, John Shell, Ransom & Randolph, Toledo, Ohio, and John Timmermans and Dave Turpin, Iowa City.* The continuing study of hygroscopic expansion resulted in the development of newer investments. Hygroscopic investments can be controlled by the amount of water added to the setting investment up to a critical point. The purpose of this study was to ascertain if fixed bridges could be cast in one piece, using hygroscopic investment accurately to control the expansion and give a measurable fit on a steel die* with two crowns of a known taper. A consistent procedure was employed, using a mechanical wax-pattern former, vacuum investing, and standardized instruments for measuring materials. Controlled variables included the water/powder ratio, spatulation, type of pattern wax, setting temperature, sprue design, and position of wax pattern in mold. A.D.A. type C gold was used for all castings and cleaned with acid, then placed back on dies for measurements. A shadowgraph was used to determine the amount of expansion. Data were obtained from seventy-five one-piece, three-unit castings. Variation of expansion was calculated for type of wax, change in the spatulation, w/p ratio, and setting temperatures. Quality of casting was affected by sprue design and position of pattern in mold. The force of hygroscopic expansion did not completely overcome the resistance of hard inlay wax. The data indicated that the casting of a three-unit, one-piece bridge is feasible. Two aids to this fact would be an investment that has greater expansion properties and a softer wax manufactured especially for investments utilizing the phenomenon of hygroscopic expansion.

M37. Synthesis of 5-Methyl-2-Propoxybenzoic Acid and Its Reaction with Zinc Oxide.—*Gerhard M. Brauer and Lester Simon, National Bureau of Standards, Washington, D.C.* In a previous investigation (*J. D. Res.*, **37**:547, 1958) the reaction of *o*-ethoxybenzoic acid and zinc oxide has been reported. In order to obtain a cement that may improve solubility characteristics, 5-methyl-2-propoxybenzoic acid (MPBA) was synthesized by the following procedures:



* Invar.

MPBA hardened slowly with zinc oxide, and MPBA-eugenol mixtures set within 20 minutes. A mix of standard consistence was obtained with difficulty, since the excess zinc oxide was not readily wetted by the liquid. Improvement of the cement by the addition of a wetting agent was studied. Hardness and solubility characteristics of typical cement were reported.

M38. Environmental Factors and the Physical Properties of Zinc Oxide Impression Pastes.—*G. E. Myers, University of Michigan, Ann Arbor.* The physical properties of the zinc oxide—eugenol impression pastes may be affected by environmental factors either during storage or during mixing and making an impression. A variety of effects are seen, ranging from separation of the components to changes in setting times. The effects of storage under various temperature and humidity conditions, under low barometric pressure, and during transportation were investigated as they influence setting times, consistency, and flow. The effects of varying temperature and humidity conditions during mixing were investigated in relation to their effect on setting times, and the results were compared with the setting times determined under water at 98° F. Temperatures in the range 50° C. and above were found to be the most deleterious factor in storage, as compared with storage at —12° C., under saturated humidity conditions and at room temperatures. Changes in setting times and consistency of samples on return from a round trip by surface mail to Australia were similar to specimens of the same batch stored at room temperature. Initial and final setting times of specimens prepared at temperatures ranging from 75° to 98° F. and at relative humidity values from 50 to 97 per cent were determined. Setting times were reduced as the temperature and humidity increased. Values for initial set obtained when the specimen was immersed under water at 98° F. for 1 minute from the beginning of mixing corresponded closely with the values obtained at room conditions of 82° F. and 96 per cent relative humidity.

M39. Non-setting Zinc Oxide and Eugenol Cements.—*Harold R. Horn, New York City.* The temporary cementation of multiple-unit fixed prosthesis with an anodyne cement seems to be a beneficial procedure. Final cementation may be postponed until evidence of the success of the restoration can be evaluated. Full-arch splints and fixed bridges with many full-crown retainers tend to fit rather tightly because of the multiplicity of their tensofrictional grip on the prepared teeth. These restorations are difficult to remove after being tried in the mouth, even when no cement is applied as a luting agent. If a temporary cement that sets to any degree of hardness is used, extensive restorations may resist removal to such an extent that forces applied for removal would have the potential to fracture an abutment tooth or the appliance. Although cement may not be necessary as an adjunct to retention, it is necessary to provide a marginal seal against the ingress of saliva and bacteria. It is also of therapeutic value. The practice of mixing vaseline with zinc oxide and eugenol for this purpose has many shortcomings. There is a need for a non-setting zinc oxide and eugenol cement. Cements of this type must be formulated to retain their original consistency over an extended period and be insoluble in the oral fluids, so that they maintain a marginal seal. Formulations, based on the addition of a silicone material and neutral esters of *p*-hydroxybenzoic acid to commercial brands of zinc oxide and eugenol cement, were described and evaluated.

M40. Continued Studies on the Permeability of Cavity Liners to Certain Agents.—*M. L. Swartz, N. Chamberlin, and R. W. Phillips, Indiana University School of Dentistry, Indianapolis.* The permeability of six commercial cavity liners to certain chemical compounds (sodium fluoride, acetic acid, phosphoric acid, and sodium chloride) was investigated by measuring the rate of penetration through films of the materials. This was accomplished electrolytically. The bottoms of two glass vials were removed. The cut ends were sealed together, separated only by a film of the cavity

liner which was supported on a piece of porous glass. Distilled water was then placed in one vial and a solution of the compound to be tested in the other. Electrodes were inserted in the tube containing the distilled water. The change produced in the conductance of the distilled water as the electrolytes penetrated the films was measured and automatically recorded during a 24-hour period. Similar tests were made by substituting thin slabs of dentin for the porous glass. In tests where the lining materials contained calcium hydroxide, the conductivity measurements were augmented by chemical analysis. The data did not indicate that any of the lining materials completely inhibited the passage of the compounds tested, but they did reveal that the passage was greatly retarded. For example, when the permeability of the materials to phosphoric acid was tested, the conductivity at ½ hour ranged from 1 to 2 per cent of that observed in tests where the glass was not coated with a protective agent. At 24 hours the conductivity of the experimentals was only about 15–25 per cent of that observed in controls. The data with dentin were in agreement with those obtained on the glass, although the variation between individual results was much greater.

M41. Elastomeric Denture-Base Liners.—*D. C. Smith, Northwestern University Dental School, Evanston, Illinois, and J. F. Bates, University of Manchester, England.* The physical properties of ten lining materials were evaluated and a preliminary clinical study made. These materials included seven commercial brands of the following types: heat- and cold-curing silicones, plasticized and copolymerized heat- and cold-curing acrylics, and plasticized polyvinyl chloride/acetate. Water absorption, softness, bond strength, and tensile strength were determined. Excellent bonding was observed with the heat-cured silicone and acrylic materials, but large variations were found with the cold-curing silicones, depending on the precise method of application of the primer and the viscosity of the paste. An adhesive paste method was developed for bonding highly plasticized polyvinyl chloride liners, but the liners so produced had little practical advantage over some of the others. In practice, best results were obtained with a heat-cured silicone and a heat-cured acrylic copolymer.

M42. Bonding of Plastic Teeth to Acrylic-Resin Denture-Base Materials.—*Søren E. Sørensen and Erling Fjeldstad, Marquette University, Milwaukee, Wisconsin.* The purpose of this investigation was to determine the tensile strength for bonding of plastic teeth to various brands of self-curing denture and repair resins and heat-curing denture-base resins and to study the factors which affect the strength of the bond. Specimens were produced according to the American Dental Association's Specificaiton No. 15, Section 4 • 3 • 4, except that no wax was used for the mold preparation. Two brands of teeth, five brands of self-curing resin, and four brands of heat-curing resin were studied. Variations in bonding strength ranging from 50 to 560 kg/cm^2 were observed. Highest bonding-strength values were obtained with heat-curing resins. The surface condition of the plastic teeth was found to influence the bonding strength, particularly with the self-curing resins. Even if the ridge-lap surfaces were free from all traces of wax or other contaminants, poor bonding resulted with some self-curing resins unless the tooth surface had been exposed to a suitable solvent (ethyl acetate or monomer). The effect of solvents such as ethyl acetate, monomer, and cross-linked monomer on the ridge-lap surface of plastic teeth was illustrated and was correlated with the differences in bonding strength determined after the use of these solvents.

M43. A Quantitative Description of Polymerization to Limiting Conversion.—*Kenneth F. O'Driscoll, Villanova University, Villanova, Pennsylvania.* It is a well-known fact that when an initiating system such as dimethylaniline–benzoyl peroxide is used to polymerize vinyl monomers, the polymerization ceases at a point short of 100 per cent conversion. A quantitative explanation of this phenomenon was obtained by means of a complete development of the kinetics of the formation of polymer and disappearance of initiator, when the initiator is of a redox type. The

unexpected result of this treatment was that the limiting conversion of monomer to polymer was determined not by the absolute amounts of the peroxide and amine present at the start of polymerization but by the *ratio* of the concentrations of one to the other. Polymerization to a complete 100 per cent conversion was possible only when the ratio of amine to peroxide concentrations was equal. Slight deviations from this value led to large reductions in the limiting per cent conversions. When the peroxide was present in excess of the amine, polymerization continued beyond the predicted limiting conversion. When the reverse was true, however, additional heating produced only thermal polymerization beyond the theoretical limiting conversion.

M44. A Surface Activity Test.—*R. L. Rowen, National Bureau of Standards, Washington, D.C.* A test method was designed to gain information on the nature of the surface of the hard tooth tissue. At present it utilizes a system of *n*-butanol, water, and finely divided powders of dentin, enamel, bone ash, anorganic bone, precipitated tricalcium phosphate, and, for comparison and contrast, ferric oxide and silica. These powders are normally hydrophilic (stay in the aqueous phase), but, when certain surface-active agents are added to the system, some of the powders are preferentially wetted by the *n*-butanol (i.e., the become organophilic). This effect was dependent upon the pH, but generally, for the toothlike powders, chelate > anionic > cationic > non-ionic surface-active agents. This change is presently being interpreted as the ability of particular chemical groups to bond to the tooth surfaces in the presence of water. This method may lead to mechanisms for obtaining moisture-resistant adhesion of materials to hard tooth tissues and other solids by indicating what the chemical structure of a surface-active chelate monomer should be. It may also suggest mechanisms for the bonding between the protein helix side chains and the apatite surfaces in natural calcified tissues.

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Swartz, M. L., M40; Sweeney, E. A., 298; Szmyd, L., 148; Tamarin, A., 136; Taylor, C., 101; Taylor, D. F., M15, M16, M30; Taylor, J. B., 276; Taylor, R. G., 134; Terkla, L. G., M31; Theilade, E., 216; Thompson, R. H., Jr., 261; Thomassen, P. R., 142; Timmermans, J., M36; Tisza, V. B., 264; Trautz, O. R., 171, 172; Tullar, P. E., 105; Turesky, S., 309; Turpin, D., M36; Tuttle, L., 146; Tye, M., 27; Van Huysen, G., 38, 145, 333; Van Reen, R., 68, 69; Van Wagenen, G., 256; Venkateswarlu, P., 242; Vickers, R. A., 26; Volker, J., 306; Volker, J. F., 157; Volpe, A. R., 99; Wachtel, L. W., 243; Wade, G. W., 140, 221; Waggener, D. T., 285; Wagner, E. M., 252; Wainwright, W. W., 276; Wallace, B., 337; Walters, V. E., 292; Warren, D. W., 260; Watts, H. W., 102; Watson, M. L., 81; Weber, T. B., 149, 303; Weisberger, D., 229, 230; Wells, H., 121, 278; West, T. L., 98; Wick, J. H., M12; Wildman, J. D., 314; Wilson, C. J., M35; Winer, R. A., 61; Withier, R. E., 195; Wolcott, R. B., 58, 129, 130; Wolochow, H., 133; Won, L. C., M25; Wright, D. N., 174; Wynn, W., 52, 135; Wyshak, G. H., 229, 230; Yaeger, J. A., 90; Yap, R., 205; Yen, P. K-J., 141; Yerganian, G., 63, 188; Youden, W. J., M14; Yrastorza, J., 284; Zach, L., 305; Zackeral, W., 240; Zander, H. A., 181; Zapanta, R. R., 172; Zeldow, B. J., 213; Zimmerman, S. O., 154; Zinner, D. D., 66, 106; Zipkin, I., 225, 226.

Proceedings of the Thirty-ninth General Meeting Boston, Massachusetts, March 23, 24, 25, 26, 1961

COUNCIL PROCEEDINGS

The Council of the I.A.D.R. met on Thursday morning, March 23, 1961, and at noon on each of the following days of the meeting. Sections of the North American Division and the Dental Materials Group were represented; the British Division was unofficially represented. Dr. Holmes Knighton presided at all sessions.

A number of matters concerning the business and welfare of the Association were discussed at length and the following actions were taken:

William Sweeney and Barnet M. Levy were re-elected members of the *ad interim* committee of the Council.

George C. Paffenbarger was designated official representative of the North American Division to the Japanese Division and instructed to carry greetings to the Japanese Division.

Josse E. de Wever was appointed representative for the group to arrange transportation to the Second Oral Biology Conference to be held in Bonn, Germany, July, 1962.

Mrs. Mildred Erickson, of the American Institute of Biological Sciences, was appointed Assistant Secretary-Treasurer of the I.A.D.R.

The following were designated I.A.D.R. candidates for honorary vice-president of the 16th International Congress of Zoölogy: J. L. T. Appleton, Reider F. Sognnaes, Thomas J. Hill, Martin A. Rushton, and Jens J. Pindborg. Council nominees for the 1962 ballot were named as follows: for president, Seymour J. Kreshover; for president-elect, Dan Y. Burrill; for vice-president, Martin A. Rushton (Great Britain); for secretary-treasurer, Joseph C. Muhler; for editor (2-year term), Frank J. Orland; for publication committeeman (2-year term), David F. Mitchell; for councillors-at-large (2 to be elected), Clive I. Mohammed and Cosmo R. Castaldi.

Other nominations for the official ballot may be made by petition according to the procedure outlined in Article X, Section 2, of the Constitution.

It was pointed out that the nomination of a candidate from a division other than the North American Division is in accord with the desire to emphasize the international character of the Association. Heretofore, with the major portion of the membership in North America, candidates for officers were selected from among North American members in the interest of expeditious transaction of necessary business of the Association. Within recent years, however, membership in the divisions and sections outside North America has increased markedly, and, in the opinion of the Council, it is time that this expansion of interest is reflected in the governing body of the Association. It is fitting that the first candidate for office from an area other than North America should be a member of the largest of such divisions, the British Division.

A committee appointed to study the Edward H. Hatton Award Competition (S. Kreshover, G. Ryge, and N. Williams) advised that the regular Hatton Award Committee consider revision of the rules of the competition for the 1962 meeting. Among the matters to be considered is an additional award to be conferred on the undergraduate student presenting the best research report in competition with other undergraduates.

Another committee on awards was appointed to carry on work started by the *ad hoc* committee of the Boston meeting with the purpose of planning a program of awards and seeking funds for such a program. The committee (R. S. Manly, B. M. Levy, D. Y. Burrill, with T. J. Hill and H. C. Hodge as advisers) was instructed to report at the 1962 meeting.

The Council voted approval of a July date for the 1965 meeting in Toronto instead of the usual March dates. The American Association of Dental Schools requested a delay in making this change to allow study of the situation. The A.A.D.S. committee on meeting places (which selects the even-year sites) suggested that Miami Beach, Florida, be considered for the 1966 meeting, and the I.A.D.R. Council directed that the meeting be held there if suitable arrangements could be made.

It was voted that a Biographical Directory of I.A.D.R. members be prepared and published with the assistance of a grant of $2,900 offered by the Gies Foundation for the purpose, plus $1,000 to be obtained from some other source.

The Secretary-Treasurer and Assistant Secretary-Treasurer were directed not to supply lists of I.A.D.R. members for any commercial purpose whatever. On approval of the Secretary-Treasurer, however, the A.I.B.S. will be authorized to mail suitable material to members of the I.A.D.R., provided that the expense of such mailing is paid by the person seeking to have the material distributed.

James English, as the next president, was directed to form a liaison committee to work with similar committees of other organizations for exchange of information and views.

W. C. Hess and W. D. Armstrong, constituting a committee appointed for the purpose, presented a resolution on the matter of animal experimentation. The resolution was adopted.

Problems concerning the programs for the meetings of the Association were discussed. In view of the large number of papers submitted, it is important that the papers accepted for the program should be properly prepared and presented. It was suggested that clear instructions for authors should be prepared and enforced; that schools or sections screen papers before they are submitted or presented; that the program committee write to the dean of each dental school, urging such screening; that Councillors be urged to see to the screening of papers and abstracts; that authors have trial runs of their paper before presenting them.

On the perennial question of whether a non-author could read a paper for an author who could not attend the meeting, the Council voted "No," as it has consistently in the past.

Council voted that for the 1962 meeting the Association should provide *either* preprinted abstracts *or* published abstracts in the *Journal of Dental Research,* but *not both,* and directed that the membership be polled as to its preference.

Editor Orland explained the difficulties involved in expansion of the *Journal of Dental Research,* especially the added time, space, and money necessary to convert the *Journal* to a monthly publication. Such conversion, in the opinion of the editor, is not feasible or justifiable at this time.

It was voted that a copy of the Constitution and by-laws as revised be sent to each member of the I.A.D.R.

Dues for 1962 were set at $15 for members in North America, $7 of which is for a subscription to the *Journal of Dental Research,* and, for members outside North America, $10 with the *Journal* or $3 without the *Journal.*

Chairman Louis Grossman reported for the membership committee that 178 nominations for election to membership had been accepted and 4 lacking proper certification had been provisionally accepted. Certification was completed for the 4, and all 182 persons were nominated by the Council for election to membership. The list was posted at the registration desk.

FIRST GENERAL EXECUTIVE SESSION, THURSDAY, MARCH 23, 8:00 P.M.

A quorum was present. Doctor Knighton presided. The minutes of the 1960 meeting were approved as printed in the *Journal of Dental Research,* Volume **39,** pp. 772 ff.

G. W. Burke, D. F. Taylor, and M. G. Buonocore were appointed tellers to count ballots and report at the final executive session.

Secretary-Treasurer Burrill reported on the financial condition of the Association and on the transfer of the routine business of the Secretary-Treasurer's office to the Management Service of the A.I.B.S. The report was approved. (See supplement.)

Editor Orland reported on the conduct of the *Journal.* His report appears in the supplement.

G. Paffenbarger reported that the Dental Materials Group of the Association had voted to continue ad infinitum its yearly contribution for the support of the *Journal of Dental Research.*

Actions taken and recommendations of the Council as stated in the Proceedings of Council Meetings were reported and approved.

All members of the Board of Directors were re-elected.

MEETING OF THE BOARD OF DIRECTORS OF THE CORPORATION

The directors authorized the signing of checks as required by the Constitution and approved the Evanston State Bank and Trust Company and the Riggs National Bank of Washington as repositories of Association funds.

FINAL EXECUTIVE SESSION, SUNDAY, MARCH 26, 4:30 P.M.

A quorum was present. President Knighton opened the meeting as a session of the North American Division of the I.A.D.R. S. J. Kreshover and W. Lefkowitz were elected representatives of the I.A.D.R. to the council of the A.A.A.S. S. Wah Leung was elected representative to Section Nd of the A.A.A.S. The meeting was adjourned and immediately reconvened as a general session of the I.A.D.R.

Future meetings of the North American Division of the I.A.D.R. were announced as follows: March 15–18, 1962, St. Louis; March 21–24, 1963, Pittsburgh; March 19–22, 1964, Los Angeles; July 22–25, 1965, Toronto (dates provisional); March or April, 1966, Miami Beach (pending).

The 182 persons nominated by the Council for membership in the Association were unanimously elected. (See supplement for list.)

Approval of the following budgets and expenditures was unanimously voted:

President's fund
Public relations expense for 1962 meeting
Expense of office of Secretary-Treasurer
Toward cost of Biographical Directory, if needed
Fee as affiliate of A.I.B.S
Expenses of meeting of program committee to arrange 1962 program
Expenses of meeting of committee on awards
Costs of services by A.I.B.S. for Association for year ending April, 1961
Expenses of 1961 meeting
Petty-cash expenses of publication committeemen
Minor expenses of preparation for Oral Biology Conference II
Expenditure of excess Association funds for support of the *Journal of Dental Research*

Council action setting dues for 1962 and council nominations for the 1962 ballot were announced. Banks selected by Directors as repositories of funds were approved.

President-Elect English spoke on the need for emphasis on internationality and was instructed to improve contacts with divisions and sections in other countries to this end.

The report by T. J. Hill and W. D. Armstrong on the need for federal funds for dental research and improvement of dental health was adopted and appears in the supplement.

G. W. Burke reported for the tellers on the counting of ballots. The constitutional amendment on membership was adopted by a vote of 448 members in favor, 20 opposed, and 67 abstaining. The officers elected were named. The amendment to the Constitution and the list of officers elected appear in the supplement.

An amendment to the by-laws defining a term in the Constitution as amended was proposed and adopted. The new by-law appears in the supplement.

Procedures for election of members under the amended Constitution and by-laws were explained as follows: Nominations stating the basis of eligibility for election to membership and signed by two members of the I.A.D.R. may be sent to the A.I.B.S. office in Washington, D.C., at any time during the year. The nominations will be screened by the Assistant Secretary-Treasurer for statement of eligibility and signatures of nominators and, if complete, will be sent to the membership committee for approval. Names of approved nominees will be listed in the next regular mailing to all members of the I.A.D.R. After 90 days, unless written objection by a member is received within that period, the nominee will be considered elected and becomes a member of the I.A.D.R., provided that he accepts the election and pays the annual dues. This procedure will allow the membership committee time for careful consideration of the nominations.

On behalf of the Association the President thanked John B. Macdonald and the members of his local arrangements committee, as well as Vincent F. Lisanti and members of his Public Relations Committee. Mr. Harold Osborne of the A.I.B.S. was also thanked for his public relations work at the meeting. Warm thanks went to the dental students and dental hygiene students of Boston who assisted at the meeting.

The Secretary-Treasurer read the names of members who had died during the year, and the assembly paid tribute to their memory.

The newly elected officers were installed and the chair was turned over to the new president. President English expressed his thanks to the past officers and to all who had functioned to make the meeting a success and adjourned the meeting *sine die*.

A. REPORT OF THE EDITOR

It has become customary over the years for the editor of the *Journal of Dental Research* to present an annual report to the Association. As editors age, these reports become shorter and shorter. Since editors age very rapidly, I've learned, this report will accordingly be short, but, I trust, informative.

Since that first issue in 1919, the *Journal* has been growing—sometimes rapidly, sometimes slowly, and sometimes merely maintaining itself, and this has almost always been a true reflection of the times.

Any journal anywhere these days is expensive, though a very useful enterprise if it fulfils its objectives. The *Journal of Dental Research,* especially with the increased number of pages, is an enterprise running in the one-twentieth of a million dollar level per annum. (In point of fact, the amount paid in 1960 to the University of Chicago Press for 1,268 manuscript pages was $52,304.48. The previous publisher in 1959 received $55,304.48 for 1,240 manuscript pages.)

Its objective is clearly to serve the Association specifically, and science in general, by publishing as many worthy manuscripts as possible each year. It would be the profoundest hope of the editor, the printer, and, of course, every author if his or her manuscript could be published within weeks rather than months. But with the vast number of manuscripts, some worthy and some unworthy, inundating the editor's desk each week and every week, it is a truism that a considerable lag period

must and does exist. In point of fact, it averages close to 8 months at present. During the year 1960, 192 manuscripts were received. The number 425 was stamped on a paper received the second day of January, 1960, and number 617 was stamped on another coming in late on December 31. (These are the sequence of manuscript numbers that are being stamped on each manuscript since I have been editor.) Of this 192, 123 were published in 1960; 87 were revised in a minor or major manner; 20 short notes were published, 9 of which were revised, some from longer but inadequate manuscripts.

All letters to the editor, whether readily acknowledged or not, are appreciated, and appropriate action is taken thereon, either immediately or at whatever proper time may be called for in each individual case. There have been unfortunate delays in getting certain papers published but these have been beyond our immediate control. Nevertheless, we shall keep trying to produce a better *Journal* and have it out sooner, but we need your help.

The item which brought forth the most letters, and all in the affirmative, was the matter that there be published a new biographical directory of Association members. The first and last of this kind of compilation appeared in the *Journal* of October, 1952. We plan to change the color of the cover each volume. It was a pastel blue; it is now a strawberry maroon, and I am open to suggestions for next year—lavender, for the color of dentistry, leads at present.

I wish profoundly to thank that portion of the Advisory Editorial Board which has served silently but significantly in reviewing manuscripts for the past 2 years. I also wish to thank the several unnamed authorities in specific fields consulted by board members and by the editor directly. Members of the Advisory Editorial Board whose term of office terminates as of this moment are Gerald J. Cox, Milton B. Engel, Robert M. Grainger, John Haldi, Ronald L. Hartles, Henry M. Leicester, Aaron S. Posner, Marshall L. Snyder, Wendell L. Wylie, and Seymour H. Yale. Our thanks also go to John B. Macdonald, who is completing his good work on the Publication Committee and as Associate Editor. Special thanks go to Dan Y. Burrill, who as Secretary-Treasurer has been a geographically close adviser. It may be noteworthy to mention that all these members, as well as the others still serving and including the Editor, serve without remuneration except for the satisfying knowledge that we are trying to produce a scholarly *Journal*.

During the past year these funds have been received by the Treasurer and credited to the *Journal* account: U.S.P.H.S., $3,000; Colgate-Palmolive Company, $4,000 for library subscriptions. The following Supporting Associates have contributed $100 or more: The Acralite Company, New York; Julius Aderer, Incorporated, New York; Amalgamated Dental Company, Ltd., London; Astra Pharmaceutical Products, Inc., Worcester; Block Drug Company, Incorporated, Jersey City; Bristol-Myers Company, New York; L. D. Caulk Company, Milford; Coe Laboratories, Incorporated, Chicago; Colgate-Palmolive Company, Jersey City; Columbia Dentoform Corporation, New York; Crescent Manufacturing Company, Chicago; Dentists' Supply Company of New York, York; Engelhard Industries, Incorporated, Newark; Hanau Engineering Company, Incorporated, Buffalo; J. F. Jelanko and Company, Incorporated, New York; H. D. Justi and Sons, Incorporated, Philadelphia; Kerr Manufacturing Company, Detroit; Eli Lilly and Company, Indianapolis; Lorvic Corporation, St. Louis; Midwest Dental Manufacturing Company, Chicago; J. M. New Company, Hartford; Precious Metals Research Works, Incorporated, New York; Procter and Gamble Company, Cincinnati; Ritter Company, Incorporated, Denver; Schering Corporation, Bloomfield; Squibb Institute for Medical Research, New Brunswick; Surgident, Ltd., Los Angeles; S. S. White Dental Manufacturing Company, Incorporated, Philadelphia; Upjohn Company, Kalamazoo; and, last but not least, the Dental Materials Group, I.A.D.R., the Chicago section of the I.A.D.R., and an anonymous member of the Dental Materials Group.

B. FINANCIAL STATEMENTS FOR THE YEAR ENDED DECEMBER 31, 1960

March 20, 1961

International Association
for Dental Research

GENTLEMEN:

The accompanying statements of the International Association for Dental Research, showing the Receipts and Disbursments of the General Fund, *Journal of Dental Research* Fund, and the Endowment Fund for the Year Ended December 31, 1960, to the extent that they report the custody of Dr. Dan. Y. Burrill, have been reviewed and the respective items traced into the related bank statements. No other verification was made of receipts from members for dues and subscriptions, and no physical examination was made of the U.S. government bonds, shown in the Endowment Fund. Such other procedures of verification have been followed as I considered necessary in the circumstances.

In my opinion, the accompanying statements fairly present the receipts and disbursements of these

three Funds of the International Association for Dental Research, to the extent that they are under the custody of Dr. Dan Y. Burrill, on a cash basis, consistent with the procedures followed in prior years.

Signed
DALE H. TAYLOR
Certified Public Accountant

GENERAL FUND#

Receipts and Disbursements for Year
Ended December 31, 1960

Fund balance January 1, 1960			
On deposit at State Bank and Trust Company, Evanston, Illinois		$17,565.98	
Less, due Petty Cash		(27.26)	
Total Fund Balance			$17,538.72
*Receipts:**			
Subscriptions†		$ 4,001.91	
Dues‡		3,874.00	
Registration Fees and Sale of Banquet Tickets, Annual Meeting		5,362.40	
Miscellaneous		36.41	
Total Receipts			13,274.72
			$30,813.44
*Disbursements:**			
Printing and Distributing Journal C. V. Mosby Co.	$ 7.00		
University of Chicago§	5,586.00	$ 5,593.00	
Mailing operations, postage and printing		222.05	
Gratuity to Secretary-Treasurer		500.00	
Annual Meeting expense			
Meeting facilities, banquets, and luncheons	$1,758.74		
Programs, badges, etc.	679.50		
Abstracts	1,209.25		
Novice Award	100.00		
Projection and Public Address	1,028.00		
Other Expenses	289.00	5,064.49	
University of Chicago for publishing abstracts of 1960 program		1,000.00	
Subsidy transfer to *Journal*		5,000.00	
Office expenses			
Clerical and professional	$ 151.20		
Other	32.64	183.84	
Travel expense, committees and officers		336.23	
A.I.B.S. affiliate dues		100.00	
Expended for American Institute of Biological Sciences (see contra above)		33.52	
Total disbursements			18,033.13
Fund Balance, December 31, 1960‖			
On deposit at State Bank and Trust Company, Evanston, Illinois		$12,945.48	
Less, due Petty Cash		(165.17)	
Total Fund Balance			$12,780.31

* Receipts include only those items for which Dr. Dan Y. Burrill is held directly responsible. After March, 1960, all subscriptions and dues for the year 1961 were delivered to the custody of the American Institute of Biological Sciences. None of the disbursements for services performed for the period ending December 31, 1961 have been included in these disbursements. A separate accounting will be made by the above named custodian.

† The subscriptions apply to various years as follows: 1959, and prior—$161.00; 1960—$3,696.00; 1961—$17.00.

‡ The dues apply to various years as follows: 1959, and prior—$191.46; 1960—$3,807.45; 1961—$3.00.

§ No invoices for services since July, 1960, have been received from the University of Chicago. Dr. Orland reported that, as of December 31, 1960, there were no outstanding obligations due the University of Chicago from this organization.

‖ In addition to the Cash and other assets shown, the Association owns equipment which cost $577.19. There have been no additions during the year.

ENDOWMENT FUND#

Receipts and Disbursements for Year
Ended December 31, 1960

Balance in Savings Account, State Bank and Trust Company, Evanston, Illinois		$ 642.89
Receipts:		
Interest from Savings Account to December 31, 1960	$ 19.72	
Interest from U.S. Government bonds	41.40	
Total Receipts		61.12
Balance in Savings Account (credited or to be credited), State Bank and Trust Company, Evanston, Illinois		$ 704.01

U.S. GOVERNMENT BONDS#

Series K Bonds	$ 1,500.00	
Series G Bonds, now matured	100.00	
Series E Bonds, dated August 1959, par $100		
At cost	75.00	
Total		$ 1,675.00

JOURNAL OF DENTAL RESEARCH FUND

Receipts and Disbursements for Year
Ended December 31, 1960

Fund Balance, January 1, 1960:			
On deposit at State Bank and Trust Company, Evanston, Illinois			$10,262.66
Receipts:			
Supporting Associates		$ 1,850.00	
William J. Gies Foundation	$1,500.00		
U.S.P.H.S., N.I.H. Grant	4,000.00	5,500.00	
From General Fund		5,500.00	
Sale of back issues		1,001.54	
Total Receipts			13,351.54
			$23,614.20
Disbursements:			
C. V. Mosby Co. for extra pages		$ 1,181.25	
C. V. Mosby Co. for back issues (From Gies Foundation)		2,000.00	
University of Chicago, Services to *Journal of Dental Research*, through July, 1960§	$ 4,212.89		
Subsidy toward publication	10,000.00	14,212.89	
Advanced to D. F. Mitchell for Petty Cash expenditures		19.07	
American Association of Dental Editors—Dues		10.00	
Total disbursements			17,423.21
Fund Balance, December 31, 1960			
On deposit at State Bank and Trust Company, Evanston, Illinois			$ 6,190.99

The accompanying statements have been prepared on a Cash Basis and do not reflect any obligations of the Association which may not have been paid at December 31, 1960.

C. STATEMENT FOR TRANSMISSION TO SENATE AND HOUSE COMMITTEES ON APPROPRIATIONS

The International Association for Dental Research is an organization of over 1,100 dental scientists and research investigators. At its thirty-ninth annual meeting in Boston, March 23–26, 1961, the Association held the largest meeting in its history. Over 380 reports were presented on biological and physical studies relating to oral health and disease. The complete program of the meeting is appended to this statement.

The Association recognizes the great contribution which the federal government has made in support of many of these studies. It also recognizes the government's contribution toward the production of scientists qualified to conduct dental research.

In view of the magnitude of the dental problem in the United States, the Association calls attention of the Congress to the urgent needs of the nation and to the benefits for the public that result from the use of federal funds. The Association urges that this program be intensified and broadened.

The Association urges specifically that substantial increases be made by the Congress in the appropriations to support the research and training programs administered by the National Institute of Dental Research.

The Association requests that this statement, with the appended program, be made a part of the Record.

D. AMENDMENTS TO THE CONSTITUTION AND BY-LAWS

Constitutional Amendment

Article III, Membership.—Sections 2, 3, and 4 are repealed, and in their place the following substituted:

Section 2, Election.—Any person who is eligible for election to membership under Article III, Section 1, who shall have been nominated in writing in due form by two members of the Association, and who shall have been certified by the Secretary-Treasurer or Assistant Secretary-Treasurer and approved by the membership committee shall become a member of the Association a reasonable time after the notice of his nomination has been mailed to the members of the Association, provided no member of the Association gives notice of objection to the election within such reasonable time.

Article III.—Section 5 shall be renumbered Section 3.

Amendment to By-Laws

Section H.—Definitions. Add paragraph 3 as follows:

3. The term "reasonable time" in Article III, Section 2, of the Constitution as amended means 90 days.

E. ELECTIONS TO MEMBERSHIP, MARCH 25, 1961

North America.—A. Bruce Ady, *Portland, Ore.*; Benjamin H. Amden, *Boston, Mass.*; Harold C. Askew, *Birmingham, Ala.*; Alfred T. Baum, *Los Angeles, Calif.*; Lloyd Baum, *Loma Linda, Calif.*; Norman E. Boucher, Jr., *Milwaukee, Wis.*; Norbert J. Burzynski, *St. Louis, Mo.*; Charles A. Clark, *Pittsburgh, Pa.*; David Cohn, *Kansas City, Mo.*; William K. Collett, *Pittsburgh, Pa.*; Charles William Conroy, *Houston, Tex.*; Ingeborg Constantinos, *Washington, D.C.*; Stephen F. Dachi, *Indianapolis, Ind.*; Milton H. Dalbow, Jr., *Springdale, Pa.*; Jack Gilbert Dale, *Boston, Mass.*; Angelo D'Amico, *Stockton, Calif.*; Douglas O. deShazer, *Iowa City, Ia.*; Thomas Reed Dirksen, *Brooks AFB, Tex.*; Leon Dogon, *Boston, Mass.*; Bernard R. Duke, *Wauwatosa, Wis.*; Harold L. Dute, *Los Angeles, Calif.;* Edward Eigen, *New Brunswick, N.J.*; William Kent Elwood, *Detroit, Mich.*; Richard Paul Elzay, *Indianapolis, Ind.*; Benjamin Ershoff, *Culver City, Calif.*; Will Faermark, *Studio City, Calif.*; Elliott M. Feinberg, *Brooklyn, N.Y.*; Peter Dante Ferrigno, *Washington, D.C.*; Harry Blair Fleming, *Richmond, Va.*; Donald E. Foley, *Milwaukee, Wis.*; Stephen P. Forrest, *St. Louis, Mo.*; Marion D. Francis, *Cincinnati, Ohio;* Arthur R. Frechette, *Bethesda, Md.*; *Stanley* Marion Garn, *Yellow Springs, Ohio;* Ronald Joseph Geczik, *New Brunswick, N.J.*; William A. Gibson, Jr., *Boston, Mass.*; John E. Gilster, *St. Louis, Mo.*; John F. Glenn, *Milford, Del.*; John A. Gray, III *Cincinnati, Ohio;* Emil S. Griffiths, *Albany, N.Y.*; Richard C. Grossman, *Bethesda, Md.*; Berge Hampar, *New York, N.Y.*; Bertram L. Hanna, *Bethesda, Md.*; Robert S. Harris, *Cambridge, Mass.*; James D. Harrison, *St. Louis, Mo.*; Arthur Bernard Hattler, *Bryn Mawr, Pa.*; Jess Hayden, Jr., *Loma Linda, Calif.*; Stanley P. Hazen, *Rochester, N.Y.*; Fred A. Henny, *Detroit, Mich.*; P. W. Herrick, *Iowa City, Ia.*; Fred Herzberg, *Van Nuys, Calif.*; Sheldon Holen, *New York, N.Y.*; Harold R. Horn, *New York, N.Y.*; Marjorie Houston, *Chicago, Ill.*; Clyde E. Ingersoll, *Buffalo, N.Y.*; Robert J. Isaacson, *Minneapolis, Minn.*; Leif B. Johannessen, *Altadena, Calif.*; Wallace W. Johnson, *Iowa City, Ia.*; Harold V. Jordan, *Bethesda, Md.*; Zack Kasloff, *Winnipeg, Canada*; Douglas Ward Kerr, *San Francisco, Calif.*; Richard C. Kestenbaum, *New Brunswick, N.J.*; Enrique Kiguel, *Washington, D.C.*; Jacques Kohl, *Rochester, N.Y.*; Theodore I. Koulourides, *Birmingham, Ala.*; Leonard M. Kraske, *Great Lakes, Ill.*; Od Krikadorn, *Birmingham, Ala.*; Donald Charles Kroeger, *Houston, Tex.*; Lawrence Landry, *Chicago, Ill.*; Robert H. Loving, *Great Lakes, Ill.*; Melvin R. Lund, *Loma Linda, Calif.*; Ralph H. McCormick, *Yeadon, Pa.*; Harry Maeth, *Mosinee, Wis.*; Rollin Mallernee, *Loma Linda, Calif.*; Peter M. Margetis, *Washington, D.C.*; Fujio Miura,

Chicago, Ill.; Woodrow S. Monica, *Orange, N.J.*; George Mumford, *Indianapolis, Ind.*; Rafael Nadal, *San Juan, P.R.*; Samuel Charles Nadler, *Forest Hills, N.Y.*; John Nalbandian, *Boston, Mass.*; Frank H. Nealon, *Cleveland, Ohio*; William J. O'Brien, *New York, N.Y.*; Pat O'Day, *La Puente, Calif.*; William Foster O'Meara, *Iowa City, Ia.*; James E. Overberger, *Morgantown, W. Va.*; Jasvant Parikh, *Washington, D.C.*; Richard B. Parker, *Portland, Ore.*; Patricia A. Parsons, *St. Louis, Mo.*; J. Georges Perreault, *Quebec, Canada*; John F. Prichard, *Fort Worth, Tex.*; Gertrude Quigley, *Boston, Mass.*; Lewis James Rattner, *Redwood City, Calif.*; Charles M. Reeve, *Rochester, Minn.*; Marvin Revzin, *Detroit, Mich.*; Virgil J. Richter, *Jersey City, N.J.*; Robert M. Ricketts, *Pacific Palisades, Calif.*; Anthony A. Rizzo, *Bethesda, Md.*; Mario Santos Rodrigues, *Washington, D.C.*; Buron Rosan, *Philadelphia, Pa.*; William R. Sabes, *St. Louis Park, Minn.*; Ernest A. Sahs, *Iowa City, Ia.*; Erwin Michael Schaeffer, *Minneapolis, Minn.*; Albert Schatz, *Philadelphia, Pa.*; Harold E. Schnepper, *Rialto, Calif.*; Carlos F. Schuessler, *Brooks AFB, Tex.*; Leon Segat, *Ann Arbor, Mich.*; Knut A. Selvig, *Rochester, N.Y.*; Harry B. Sheldon, *Washington, D.C.*; Irwin I. Ship, *Philadelphia, Pa.*; William R. Shiller, *Great Lakes, Ill.*; Sol Silverman, Jr., *San Francisco, Calif.*; Theodore H. Simpson, *Washington, D.C.*; Roger F. Sisca, *Allison Park, Pa.*; Richard Snyder, *Tucson, Ariz.*; Sigmund Sydney Socransky, *Boston, Mass.*; Warren Roger Sorg, *Flemington, N.J.*; James McGranahan Stewart, *West Palm Beach, Fla.*; Gerald D. Stibbs, *Seattle, Wash.*; James John Stickley, *Brookline, Mass.*; Ellery C. Stowell, *Long Beach, Calif.*; Charles F. Sumner, III, *Washington, D.C.*; Yang Gook Sunoo, *Chicago, Ill.*; Gertrude Tank, *Corvallis, Ore.*; James B. Taylor, *Corona Del Mar, Calif.*; Louis G. Terkla, *Portland, Ore.*; Robert H. Thompson, Jr., *New York, N.Y.*; Joseph Tonzetich, *Jersey City, N.J.*; Irma Tuck-Weiss, *New York, N.Y.*; John L. Tucker, *Philadelphia, Pa.*; Coleman Reed Tuckson, Jr., *Washington, D.C.*; Robert A. Uchin, *Fort Lauderdale, Fla.*; Donald E. Van Scotter, *Wood, Wis.*; Anthony Volpe, *Jersey City, N.J.*; George Wesley Wade, *Washington, D.C.*; Harvey Webb, Jr., *Washington, D.C.*; Frederick William Wertheimer, *Bethesda, Md.*; Roy Edward Wuthier, *Boston, Mass.*; Shin Hyun Yoon, *Boston, Mass.*; S. Jerome Zackin, *Lynn, Mass.*; J. Eugene Ziegler, *Los Angeles, Calif.*; Stuart O. Zimmerman, *Chicago, Ill.*

GREAT BRITAIN.—William Henry Bowen, *London;* Ian Arthur Findlay, *Belfast;* Sidney Francis Fish, *London;* Naylor Malcolm Neville, *London;* John Albert Pedler, *London;* Denis Charles Alec Picton, *Surrey;* Norman Randall Thomas, *London.*

ISRAEL.—David Gutman, *Haifa;* Itzhac Gedalia, *Jerusalem;* Edith Koyoundjisky, *Jerusalem;* Anzelm Langer, *Tel-Aviv;* Jacob Lewis-Epstein, *Jerusalem;* Julius Michmann, *Jerusalem;* Kurt A. Rosenzweig, *Jerusalem;* Ino Sciaky, *Jerusalem;* Siegfried Seidner, *Tel-Aviv;* Mario Ulmansky, *Jerusalem.*

AUSTRALIA.—J. H. Anker, *Penrith;* Percy W. Arkle, *Queenstown;* M. J. Barrett, *Adelaide;* T. Brown, *Adelaide;* Peter B. Cadell, *Dunedin, N.Z.*; J. A. Cran, *Adelaide;* P. G. Dellow, *Adelaide;* Barrie Roderick D'Arcy Gillings, *Sydney;* C. J. Griffin, *Sydney;* A. M. Horsnell, *Adelaide;* J. Eustace L. Jeffrey, *Victoria;* B. J. Kruger, *Brisbane;* N.D. Martin, *Sydney;* B. G. Radden, *Adelaide;* P. C. Reade, *Adelaide.*

OTHER COUNTRIES.—Finn Protorius Clausen, *Denmark;* Paul H. Erdle, *Germany;* Erik Hjorting-Hansen, *Denmark;* Kalervo K. Koivumaa, *Finland;* Cid Menegale, *Brazil;* Mauricio Moscovici, *Brazil;* Hans Peter Philipsen, *Denmark;* Paul E. Sutter, *Switzerland.*

F. MISCELLANEOUS

1. *Statistics:*

Current membership		Attendance at 1961 Meeting	
North American Division	888	Members	396
Others	209	Non-members	410
		Guests	89
Total	1,097	Total	895

2. *Officers elected for 1961–62:*

President	James A. English
President-Elect	Seymour J. Kreshover
Vice-President	Dan Y. Burrill
Secretary-Treasurer	Joseph C. Muhler
Publication Committeeman (two year term)	Richard S. Manly
Councillors-at-large	Josse de Wever, Clifton O. Dummett

Ascorbic Acid Plasma Levels and Gingival Health in Personnel Wintering Over in Antarctica

*MAX J. PERLITSH, ARNE G. NIELSEN, and WILLIAM R. STANMEYER**

Dental Branch, U.S. Naval Medical Research Laboratory, U.S.N. Submarine Base, New London, Connecticut

Studies have shown that animals exposed to the stress of extreme cold have an increased need for ascorbic acid.[1–3] The objective of this paper is to present the relationship between ascorbic acid plasma levels and gingival health as studied in men exposed to the stress of wintering over in Antarctica during the International Geophysical Year, 1957–58. Gingival tissue was selected as an indicator of tissue health, since it seems to reflect ascorbic acid deficiencies.

EXPERIMENTAL METHOD

This study was based on the monthly clinical examination, by the same investigator, of 26 men ranging in ages from twenty-one to thirty-seven years. The experimental group of 26 men was composed of 13 outdoor workers and 13 indoor workers. The age range of the outdoor group was twenty-one to thirty-five years, with an average age of 27.6 years. The age range of the indoor group was twenty-one to thirty-seven years, and the average age was 28.4 years.

Beginning in January, 1958, and until November, 1958, the subjects of the study volunteered 30 cc. of blood monthly for routine hematologic and blood chemistry analyses.†

Ascorbic acid determinations were made on the plasma of each blood sample taken from all subjects on the same day before breakfast. The analyses were completed the same day that the samples were drawn. The method used was that of Roe and Kuether,[4] in which the ascorbic acid, contained in the trichloroacetic acid filtrate of the blood is oxidized to dehydroascorbic acid by shaking with activated charcoal and coupled with 2,4 dinitrophenylhydrazine to form an osazone. The resulting osazone is treated with sulfuric acid to produce a red color, which is measured photometrically.

At monthly dental appointments, intraoral colored photographs (Kodachrome) were taken of each subject. A careful clinical examination was then conducted, with particular attention given to the degree of gingival inflammation, which was recorded using modifications of the P.M.A. Index (papilla, marginal, and attached gingiva), after the method of Massler, Schour, and Chopra.[5] The criteria used for assessing

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† The specific blood studies done were: hematologies: red-cell count, white-cell count, differential, hemoglobin; hematocrit, sedimentation rate. Sera electrolytes: sodium, potassium, chloride, phosphorus, calcium. Blood chemistries: blood glucose, non-protein nitrogen, blood urea nitrogen, ascorbic acid, total protein, creatinine, uric acid, fibrinogen, prothrombin time.

the degree of gingival health were the same as those used by the latter authors. However, in all evaluations the attached gingiva (A) was not considered; only the papillary (P) and marginal (M) gingiva were scored.

A monthly health diary was kept on all men by the dental officer. This diary was designed by the Personnel Assessment Branch of the Medical Research Laboratory, U.S. Naval Submarine Base, New London, Groton, Connecticut. The diary consisted of seventy-five questions which each subject was required to answer each month. The answers to the questions provided an indication of the subjects' physical well-being and psychological adjustment. Data obtained from the health diary included a month-

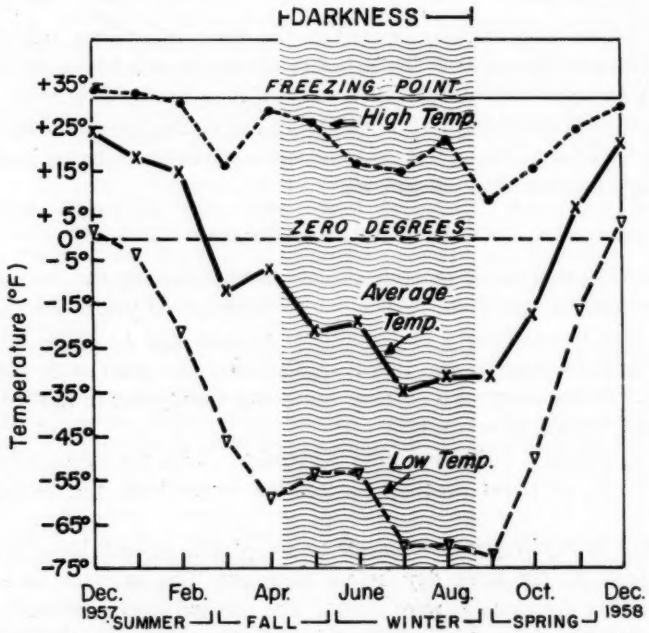

FIG. 1.—High, low, and average monthly temperatures recorded at Little America V, Antarctica, during the IGY, 1957–58.

ly record of weight, cigarette consumption, supplementary vitamin intake (tablets and capsules), and dietary desires and preferences.

Daily outdoor temperatures were provided by meteorologists of the U.S. Department of Commerce, Weather Bureau. These temperature readings served as a basis for establishing the possible effects that temperature variation had upon ascorbic acid plasma levels. The high, low, and average monthly temperatures recorded at Little America V are summarized in Figure 1.

RESULTS

The monthly ascorbic acid plasma level (AAPL) determinations on each subject (expressed in mg per cent) were tabulated (see Table 1). From these data two average AAPL's were calculated, one for the group at the end of each month and one for the individual at the end of the eleven-month period.

It is obvious at a glance that the average individual AAPL's of the outdoor group were generally lower than those of the indoor group. This table also shows that the average individual AAPL's for the year of the indoor group ranged from approximately 0.6 to 1.2 mg per cent (see *left side*, Fig. 2). This range falls within what is generally accepted as normal. However, the average individual AAPL's for the outdoor group over the same period ranged from approximately 0.5 to 0.7 mg per cent, which is generally accepted as low normal.

In order to examine the reliability of the differences between the two groups, a

TABLE 1

MONTHLY ASCORBIC ACID PLASMA LEVEL (AAPL) DETERMINATIONS (EXPRESSED IN MG PER CENT)

Subject	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Av. Indiv. AAPL
	Indoor Group											
1.....	0.65	0.62	0.60	0.65	0.65	0.60	0.52	0.50	0.65	0.53	0.55	.593
2.....	0.87	0.85	0.90	0.86	0.85	0.80	0.75	0.83	0.75	0.73	0.90	.826
3.....	0.80	0.85	0.92	0.87	0.85	0.80	0.80	0.85	0.95	0.93	0.95	.870
4.....	0.83	0.80	0.88	0.92	0.95	0.95	0.90	0.85	0.80	0.85	0.85	.870
5.....	0.92	0.90	0.87	0.88	0.85	0.85	0.88	0.85	0.80	0.82	0.95	.870
6.....	0.96	0.90	0.91	0.85	0.87	0.85	0.80	0.85	0.81	0.85	0.95	.873
7.....	0.95	0.85	0.87	0.90	0.92	0.95	0.90	0.94	0.95	0.93	0.95	.919
8.....	0.90	0.95	0.90	0.95	0.90	0.95	0.97	0.95	0.90	0.95	0.87	.926
9.....	0.94	0.93	0.95	0.95	0.90	0.95	0.90	0.90	0.95	0.97	1.10	.952
10.....	1.05	1.05	0.95	0.87	0.85	0.85	0.81	0.85	1.10	1.00	1.10	.953
11.....	1.10	1.10	0.97	0.98	0.93	0.87	0.80	0.85	0.95	0.92	1.10	.961
12.....	1.20	1.25	0.95	0.97	0.95	1.10	1.25	1.10	1.20	1.10	1.15	1.11
13.....	1.10	1.05	1.10	1.25	1.10	1.20	1.20	1.25	1.10	1.20	1.25	1.16
Monthly group average AAPL.	.944	.931	.905	.915	.890	.902	.883	.890	.916	.906	.975	.913
	Outdoor Group											
14.....	0.67	0.65	0.60	0.60	0.60	0.55	0.50	0.46	0.45	0.43	0.45	.541
15.....	0.67	0.60	0.65	0.60	0.64	0.60	0.56	0.46	0.45	0.40	0.45	.553
16.....	0.65	0.65	0.68	0.65	0.60	0.61	0.53	0.45	0.47	0.45	0.40	.558
17.....	0.75	0.70	0.70	0.72	0.60	0.55	0.50	0.45	0.45	0.40	0.43	.568
18.....	0.72	0.70	0.70	0.63	0.65	0.60	0.51	0.46	0.48	0.45	0.40	.573
19.....	0.74	0.75	0.70	0.75	0.63	0.60	0.55	0.43	0.40	0.43	0.40	.580
20.....	0.65	0.60	0.65	0.65	0.61	0.62	0.58	0.51	0.50	0.53	0.55	.586
21.....	0.75	0.72	0.75	0.70	0.65	0.56	0.55	0.49	0.46	0.45	0.40	.589
22.....	0.75	0.70	0.80	0.75	0.71	0.66	0.65	0.51	0.50	0.50	0.45	.634
23.....	0.77	0.80	0.75	0.75	0.65	0.60	0.57	0.52	0.55	0.50	0.55	.637
24.....	0.75	0.77	0.70	0.70	0.62	0.61	0.60	0.61	0.60	0.62	0.65	.657
25.....	0.85	0.80	0.75	0.70	0.72	0.70	0.65	0.60	0.57	0.55	0.50	.672
26.....	0.84	0.80	0.84	0.80	0.75	0.71	0.65	0.50	0.52	0.55	0.50	.678
Monthly group average AAPL	.735	.711	.713	.692	.648	.613	.569	.496	.492	.482	.472	.602

statistical analysis of the data was performed.* The use of an experimental design which involves successive measurements of the *same* subjects necessitates the utilization of statistical techniques that take into account the effects of correlation between the monthly measurements. Consequently, an analysis-of-variance technique designed for this purpose was employed.[6] By this method, a stringent statistical test of the significance of the differences between the indoor and outdoor groups, in terms of varying ascorbic acid plasma levels, over an eleven-month period was provided. This analysis is presented in Table 2.

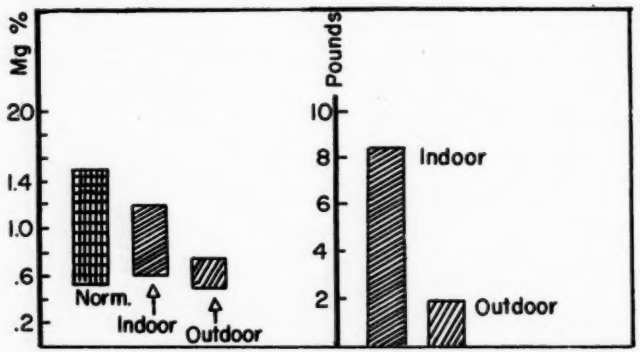

FIG. 2.—(*Left*) Comparison of the range of average AAPL's of indoor-outdoor groups with normal AAPL range. (*Right*) Comparison of average individual weight gained by indoor-outdoor groups.

TABLE 2

VARIANCE ANALYSIS OF ASCORBIC ACID PLASMA DATA FOR INDOOR-OUTDOOR GROUPS

Source of Variation	Sum of Squares	Degree of Freedom	Mean Squares	*T* Ratio	*P*
A. Between groups	69,644.63*	1	69,644.63	60.43	<0.001
B. Between subjects	27,657.43	24	1,152.39	.....	.....
C. Between months	7,516.41	10	751.64	257.41	<0.001
D. Interaction: groups×months	7,497.18	10	749.72	256.75	<0.001
E. Interaction: total subjects × months	701.26	240	2.92	.....	.....
Total	113,016.91	285	369.55	.....	.....

* Each raw score was multiplied by a constant (100) to eliminate decimals. This manipulation does not, of course, affect the *F* ratios.

Line A in Table 2 examines the question, "Do the mean ascorbic acid plasma levels differ between the indoor and outdoor groups over the eleven-month period?" The answer is "Yes," as the odds are less than one in a thousand for the differences being accounted for by chance. Line B answers the question, "Are there systematic differences over the subjects of both groups?" The obvious answer is "Yes." This step gives preparatory data for interpretation of lines C and D. Line C examines statistically the question, "Considering both indoor and outdoor groups together, do the AAPL's

* Dr. Benjamin Weybrew, head, Psychological Assessment Branch (NMRL).

differ from month to month," The answer is "Yes," as the odds are again less than one in a thousand for the difference being accounted for by chance. Line D tests the most important hypothesis, examining the question, "Does the difference between the two groups change significantly from month to month?" In this case, once again, the answer is "Yes," as the odds are less than one in a thousand for the differences being accounted for by chance.

To represent graphically the differences in AAPL's between the two groups, the monthly group average AAPL's are presented in a bar graph (see Fig. 3). This graph shows clearly the relatively high and comparatively constant AAPL's of the indoor

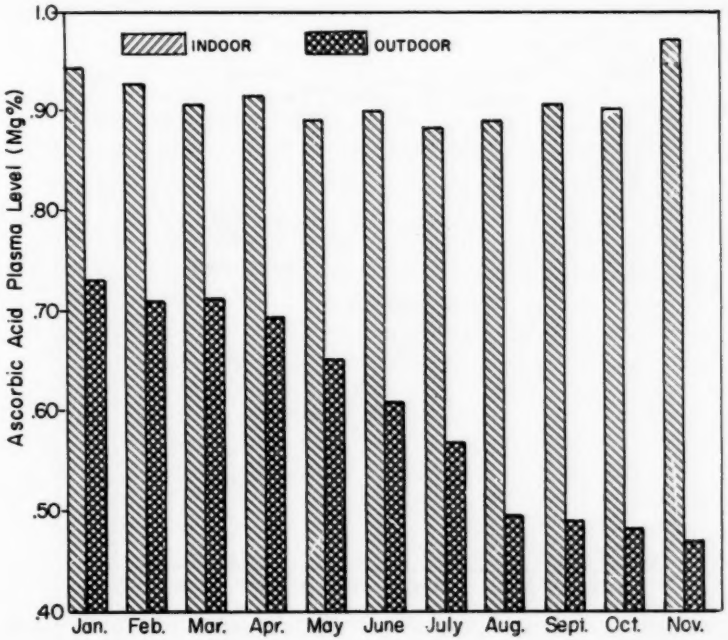

FIG. 3.—Comparison of the average monthly ascorbic acid plasma levels of the indoor and outdoor groups.

group. The AAPL's of the outdoor group were lower from the beginning and became progressively lower throughout the entire period. It is interesting to note that in Figure 4, which compares the average group AAPL's with the average monthly outdoor temperatures, the indoor group levels increased when the temperatures rose, whereas there was merely a reduction in the rate of decline for the outdoor group.

Two modifications of the P.M.A. Index were used in the analyses of the data obtained for the evaluation of gingival health. First, the number of inflamed areas, regardless of intensity, was considered. Then the data were re-evaluated and the intensity of inflammation included. Intensity was scored on a scale using normal = 0, mild gingivitis = 1, moderate gingivitis = 2, severe gingivitis = 3, and Vincent's infection = 4.

Since the number of susceptible areas varies from one subject to another, depending on the number of teeth present, the subjects were compared on a percentage basis. Table 3 presents a comparison of the indoor-outdoor individual and group average percentages on the basis of inflamed areas and intensity of inflammation. This table shows the total number of teeth of each subject, which established the total possible number of P + M areas susceptible to inflammation. Each subject's monthly P.M.A. score for the entire experimental period was analyzed to yield an individual average number of inflamed areas. These individual averages were used to compute the average

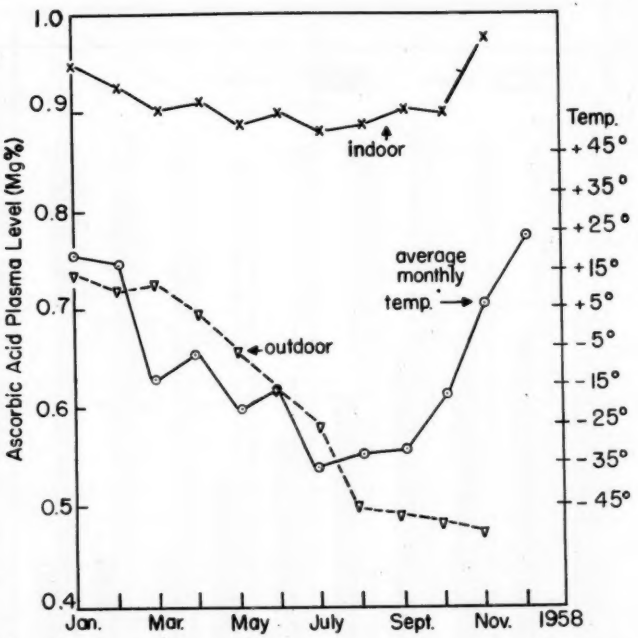

FIG. 4.—Comparison of the average monthly ascorbic acid plasma levels of the indoor and outdoor groups with the average monthly temperatures.

fraction of the total number of areas inflamed in each individual on a percentage basis, using the following formula:

$$\frac{\text{Individual average number of P + M inflamed areas}}{\text{Total individual P + M areas}} \times 100 = \text{Percentage of inflamed areas}.$$

Each subject's monthly P.M.A. score was then reanalyzed by taking the sum of the numbers assigned to each inflamed area according to the scale indicated above. These individual monthly total P + M scores were averaged to obtain the individual average P + M scores for the entire experimental period (see Table 3).

In order to compare the gingival health of both groups on a basis of intensity of inflammation, it was decided to compute a hypothetical highest possible P + M score that any subject could attain, which was represented by the product of four times

the total number of susceptible areas (total P + M) in any particular subject's mouth. The fraction of the hypothetical P + M score actually observed in each mouth was computed, using the following formula:

$$\frac{(P+M)\,\text{score}}{4\,(\text{total } P+M)} \times 100 = \text{Percentage of highest possible } (P+M)\,\text{score}\,.$$

From the individual average percentages of the highest possible P + M scores, a group average percentage figure was obtained. This figure was used to represent the average intensity of gingival inflammation for each group over the entire experimental period (see Table 3).

The data on ascorbic acid plasma levels clearly show a significant difference be-

TABLE 3

COMPARISON OF INDOOR-OUTDOOR INDIVIDUAL AND GROUP AVERAGE PERCENTAGES ON BASIS OF INFLAMED AREAS AND INTENSITY OF INFLAMMATION

Subject	Total No. of Teeth	Total P+M Areas	Average No. of Inflamed Areas	Average Per Cent Inflamed Areas	Highest Possible P+M Score	Average P+M Score (Intensity)	Per Cent of Highest Possible Score
	Indoor Group						
1	21	39	38	97	156	83	53
2	24	46	46	100	184	92	50
3	23	39	39	100	156	60	38
4	25	46	46	100	184	71	39
5	26	50	39	78	200	59	30
6	28	54	0	0	216	0	0
7	28	54	18	33	216	21	10
8	28	54	39	72	216	43	20
9	23	43	2	5	172	3	2
10	26	49	4	8	196	4	2
11	26	48	42	88	192	51	27
12	27	51	0	0	204	0	0
13	28	54	2	4	216	3	1
	333	48	24	53	.....	38	21
	Outdoor Group						
14	21	32	28	88	128	30	23
15	28	54	47	87	216	63	29
16	28	54	29	54	216	33	15
17	22	40	31	78	160	35	22
18	23	44	11	25	176	11	6
19	21	40	40	100	160	73	46
20	25	45	44	98	180	66	37
21	25	48	1	2	192	1	5
22	26	49	48	98	196	82	42
23	27	52	3	6	208	3	1
24	26	48	17	35	192	23	12
25	28	54	21	39	216	28	13
26	23	43	39	91	172	44	26
	323	46	28	62	.....	38	21

tween the indoor and outdoor groups. The question now arises, "Will this difference be manifest in the degrees of gingival health of the two groups?"

The answer, based on the number of inflamed areas in both groups, was, as shown in Table 3 and also in Figure 5, that there were 9 per cent more inflamed areas in the outdoor group, 62 per cent, as compared with the indoor group, 53 per cent. A non-parametric test applied to these two distributions showed no significant differences.[7] The answer to the above question based on the intensity of inflammation (P + M score) is that there was no significant difference in oral health of the groups, since they both presented equal scores.

A review of the monthly health diary indicates that the subjects of this study utilized supplementary vitamin sources to a varying degree. Table 4 indicates the frequency with which the outdoor-indoor subjects utilized supplementary vitamin sources.

It appears from Table 4 that the indoor group utilized supplementary vitamin sources to a greater extent than did the outdoor group. The question arose, "Did this account for the significant difference in the AAPL's between the two groups?" Grouping the frequency data in Table 4 into two groups—those never taking vitamins and those taking vitamins once a week or more often (1 degree of freedom)—resulted in a χ^2 of 0.6, reaching significance only at the 50 per cent level of confidence.

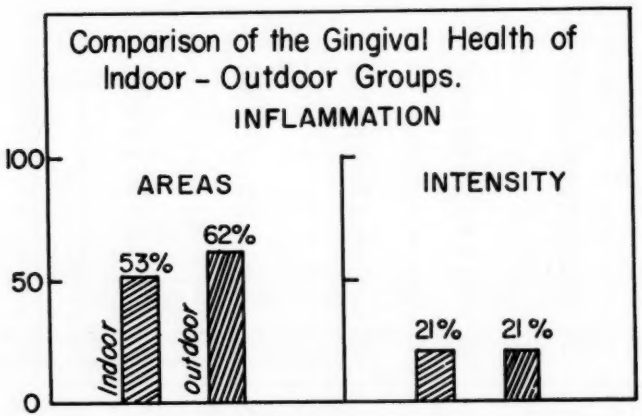

FIG. 5.—Comparison of the gingival health of indoor-outdoor groups. Group average percentages compared on basis of inflamed areas and intensity of inflammation.

TABLE 4

SUPPLEMENTARY VITAMIN HABITS OF INDOOR-OUTDOOR SUBJECTS

Frequency	Outdoor	Indoor
1. Each day	0	3
2. Three times a week	0	1
3. Two times a week	3	2
4. Once a week	3	2
5. Never	7	5

It is interesting to note in this nutritional study (*right side*, Fig. 2) that the average weight gain for the indoor group was 8.4 pounds, as compared with 1.9 pounds for the outdoor group.

The data from the health diaries indicated that both groups smoked to approximately the same extent. The indoor group averaged 18 cigarettes per man per day, and the outdoor group averaged 17 cigarettes per man per day. Lack of a significant difference in smoking between the two groups is of interest, as it has been suggested that smoking increased the need for ascorbic acid.[8]

DISCUSSION

Why was there not a larger clinical difference in the gingival health of the two groups, despite the significant difference in their ascorbic acid plasma levels? There may be several explanations for this lack of correlation. First, it may well be that a physiological difference in oral health did exist but that our gross clinical methods for evaluating it were not adequate to reflect these fine changes. It may be that the use of a tooth mobilometer to indicate changing degrees of tooth mobility[9] or the plotting of rewarming curves[10] to measure gingival circulation would give a more sensitive measure of the degree of oral health.

Second, it may be that, since the AAPL's of both groups were within the wide range of normal, that is, between 0.50 and 1.5 mg per cent, no difference in gingival health should be expected. When the plasma contains less ascorbic acid than 0.50 mg per cent, the body concentration may be designated as suboptimal. Plasma values below 0.15 mg per cent are invariably associated with clinical scurvy.[11] None of the subjects of this study had these low extremes of AAPL's, and therefore it may have been unwarranted to anticipate a dramatic difference in the gingival health of the two groups.

Third, as these results would seem to indicate, it may be that the degree of oral health and ascorbic acid plasma levels are not directly related. Perhaps, *tissue* levels of ascorbic acid are more significant than *plasma* levels. Cheraskin[8] suggests that what is needed is a laboratory test that measures ascorbic acid at the cellular level. The tissue levels of vitamin C may have been maintained at the expense of the plasma levels. Had tissue levels of ascorbic acid been recorded, using the intradermal dye decolorization test, perhaps more agreement with the oral-health findings would have resulted. Such a study is being performed in Antarctica at the present time.

It has been reported that there is a relationship between smoking and ascorbic acid requirements.[8] But in this study both groups smoked to the same extent, so that this factor can be disregarded.

The trend of the curves of the AAPL's (Fig. 4) of both groups are particularly interesting in terms of the adequacy of the vitamin C intake. The dietary sources of vitamin C available to all men at Little America were primarily from fresh-frozen citrus juices. Supplementary vitamin tablets, Hexavitamins and Decavitamins, and capsules, Viaquamin Therapeutic, containing vitamin C were also available and recommended but were taken strictly on a voluntary basis. Unfortunately, no record was kept as to how much fruit juice was consumed by each man. However, observations of the general dietary habits of the men indicated that a large majority drank at least three glasses (8 ounces per glass) of fresh-frozen citrus juice per day. The juice was

served at each meal three times a day. All other frozen sources of vitamin C were cooked prior to serving and therefore lost most of their ascorbic acid content.

Figure 4 shows that the average AAPL's for the indoor group over the experimental period started out at 0.944 mg per cent and decreased slightly as the outdoor temperature dropped, then recovered to a figure slightly above the initial level when the outdoor temperatures rose. On the other hand, the average AAPL for the outdoor group at the beginning of the study was 0.735 mg per cent, and, as the temperatures dropped, the monthly group average AAPL's declined markedly. When the outdoor temperature rose again, the monthly group average AAPL's did not improve but merely decreased at a slower rate. This leads to the conclusion that the ascorbic acid intake of the outdoor group was insufficient to meet their needs.

The fact that a significant difference in AAPL's did occur suggests that the working environment of the two groups may have been responsible. It is well documented that the caloric requirements of men exposed to extreme cold is markedly increased.[2] This fact is substantiated by observations during the period of this study. The men of the outdoor group consumed substantially more food, estimated as at least twice as much as the indoor group, but gained only one-fourth as much weight as the indoor group. It seems that, despite the increased caloric intake, their rigorous outdoor labor, in an extremely cold environment, required a greatly increased metabolic rate.

Since body nutritional requirements were higher for the outdoor group, it is reasonable to assume that the ascorbic acid requirements would also be proportionately higher. It has been estimated that the ascorbic acid intakes of both groups were approximately equal; therefore, one would expect to find a lower plasma level in the group with the higher requirement as is shown in the results of this study.

SUMMARY

A report is presented of the dental research program conducted at Little America V, Antarctica, during the International Geophysical Year, 1957–58. The program was initiated to study blood elements and their possible relationship to the etiology of cold-weather oral-health problems. The blood studies conducted are listed. This paper reports the relationship of one of the blood components—ascorbic acid plasma levels to gingival tissue health. Twenty-six men, thirteen outdoor workers and thirteen indoor workers, were the subjects of the study. Determinations of the ascorbic acid plasma levels of these men revealed a significant difference between the indoor and outdoor groups. The gingival tissue health was evaluated by observations of the relative degrees of gingival inflammation presented by the subjects of both groups, and it was found that no significant differences existed in the gingival tissue health between the groups. Several possible explanations are suggested for the lack of correlation between the ascorbic acid plasma levels and the gingival tissue health.

REFERENCES

1. Ralli, E. P. The Effect of Certain Vitamins on the Response of Normal Subjects to Cold Water Stress. Nutrition under Climatic Stress, *Proc. Nat'l Acad. Sci.*, p. 81, 1954.
2. Dugal, L. P., and Therien, M. Ascorbic Acid and Acclimatization to Cold, *Can. J. Research*, Sec. E, **25**:111, 1947.
3. Dugal, L. P. Acclimatization to Cold Environment. Nutrition under Climatic Stress, *Proc. Nat'l Acad. Sci.*, p. 70, 1954.

4. Roe, J. H., and Kuether, C. A. The Determination of Ascorbic Acid in Whole Blood and Urine through the 2,4 Dinitrophenylhydrazine Derivative of Dehydroascorbic Acid, *J. Biol. Chem.*, **147**:399, 1943.
5. Massler, M., Schour, I., and Chopra, B. Occurrence of Gingivitis in Chicago School Children, *J. Periodont.*, **21**:146, 1950.
6. Edwards, A. L. *Experimental Design in Psychological Research*, p. 288. New York: Rinehart & Co., 1950.
7. Wilcoxon, F. *Some Rapid Approximate Statistical Procedures*. New York: American Cyanimid Co., 1949.
8. Cheraskin, E., Dunbar, J. B., and Flynn, F. H. The Intradermal Ascorbic Acid Test. Part III. A Study of Forty-two Dental Students, *J. D. Med.*, **13**:135, 1958.
9. Mühlemann, H. R. Periodontometry: A Method for Measuring Tooth Mobility, *Oral Surg., Oral Med. & Oral Path.*, **4**:1220, 1951.
10. ———. Personal communication, September, 1959.
11. Goodman, L. S., and Gilman, A. *The Pharmacological Basis of Therapeutics*. p. 1720. New York: Macmillan Co., 1955.

A Study of Leptotrichia buccalis. *I. Morphology and Preliminary Observations*

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Leptotrichia buccalis, a much disputed species and even neglected in the latest *Bergey's Manual,*[1] was investigated in order to study variations in colony morphology, microscopic morphology, sensitivity to oxygen, and the best media for growth and preservation of cultures. A few papers, such as those written by Buehlmann,[2] Robin,[3] Trevisan,[4] and Miller,[5] which appeared before Kligler's[6, 7] classical experiments in 1915, dealt primarily with morphologic studies, attempts at isolation and classification. Since that time other publications by Wherry and Oliver,[8] Thjøtta, Hartmann, and Bøe,[9] Bøe,[10] Jackins and Barker,[11] and Hamilton and Zahler,[12] to name a few, have covered some of the biochemical activities of these filamentous micro-organisms. Although there were some differences among strains in these reports, such as the ability or inability of the micro-organisms to ferment lactose or raffinose, slight variations in their action on milk, etc., these should be regarded as minor strain variations. Evaluation of these differences should include the conditions such as pH, composition of the media, methods of incubation, and so forth, under which these data were obtained. Variations in conditions could produce variable effects; thus standardization of procedures is important in obtaining reproducible results.

The existence of filamentous micro-organisms in the oral cavity is a known fact, but there is no unanimity regarding their nomenclature. The confusion resulting from the use of several different names for similar species and also the application of the same name for several different species should be resolved.

Frequent mention has been made regarding the difficulty with which the oral filamentous micro-organisms were isolated and maintained on laboratory culture media, but recent papers[9–12] indicate that this is not the case with respect to *L. buccalis*. Improvements in technique and materials available have played a great part in the further study and understanding of this micro-organism.

There are many filamentous oral micro-organisms which can be observed in smears taken from the oral cavity other than the species *L. buccalis*. One finds filamentation occurring with *Fusobacterium* species and also with *Lactobacillus* species under certain conditions, but those usually revert to their "normal" appearances when cultured on laboratory media which will support their growth. There is another group of filamentous elements besides those mentioned above, which can grow either under aerobic or anaerobic environment. These, however, show dichotomous branching upon isola-

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tion and culturing in laboratory media and have been referred to as *Leptotrichia dentium* by Baird-Parker and Davis.[13] Besides these, there are other strains or species which we have observed and have not been able to isolate which can also contribute to the confusion regarding the oral filamentous micro-organisms.

We are concerned primarily in this study with the anaerobic or microaerophilic oral filamentous micro-organism commonly found in plaque material and materia alba obtained from the interproximal spaces between the teeth or in calculus around the gingival margins. We feel that this micro-organism is identical with that described by Wherry and Oliver,[8] Thjøtta *et al.*,[9] Bøe,[10] and Hamilton and Zahler[12] and the species designated *L. buccalis* by Baird-Parker and Davis.[13]

TABLE 1

BRAIN-HEART MEDIUM*

	Gm.
Calf brain (infusion)	200
Beef heart (infusion)	250
Proteose-peptone (Difco)	10
Dextrose	2
Sodium chloride	5
Disodium phosphate	2.5
Gelatin	10
Yeast extract	2
Cysteine-hydrochloride	1
Redistilled H_2O	To 1,000 ml.

* Adjusted to pH 7.3 with 1.5 ml. *N* HCl.

MATERIALS AND METHODS

Media of different composition described in a previous report[14] from this laboratory were included in this study. Alterations such as the addition of polyoxyethylene sorbitan monooleate* and soluble starch as possible growth stimulants were made. Variations of the individual ingredients in the different media were used, such as different concentrations of glucose and of the phosphate ion. Reducing agents, such as cysteine hydrochloride and sodium thioglycollate, were employed for the growth of these cultures in deep semisolid media incubated under aerobic conditions. Other nutritional supplements added in varying amounts included yeast extract, crystalline bovine serum albumin, ascitic fluid, defibrinated sheep's blood, sheep serum, saliva, and powdered enamel from extracted human teeth. Inhibiting agents such as dyes and antibiotics were also employed for some of the initial isolations from crude material.

Two simplest media from the standpoint of formulation in the laboratory were (1) brain-heart infusion broth† with yeast extract, cysteine, gelatin and inorganic salts (Table 1), and (2) tryptone broth (a pancreatic digest of casein rich in tryptophane†) with soluble starch or glucose, yeast extract, and some inorganic salts (Table 2).

The crude material for isolation of the various strains used in our study was obtained from teeth scrapings of calculus, materia alba, or the gelatinous plaque. Our earlier studies indicated that absolute aseptic methods were not necessary and that

* Tween 80.

† Difco Laboratories, Detroit, Mich.

numerous filamentous forms were found in all these materials. It was necessary only to use sterile instruments, containers, and saline diluent to minimize external contaminants. Subjects were selected from patients in the clinics and from laboratory personnel. Location of the samples in the oral cavity was not important, as it was quite easy to demonstrate the presence of these forms in wet-mount preparations observed under a phase-contrast microscope. The filamentous forms could be found in both "clean" and "unclean" mouths. Presence or absence of dental caries or other pathologic conditions did not correlate with the finding of filamentous forms on the initial isolation plates.

The inocula for the initial isolations were obtained by suspending the material from the scrapings in 0.5 ml. sterile buffered saline and grinding with a sterile glass rod (shaped to fit the bottom of the tube) to break up the clumps. A small amount of inoculum was used when either brain-heart or tryptone agar medium was used without any inhibitory agents for the initial isolations. This was obtained by means of a sterile wire-loop needle using one or two loopfuls of crude suspension and spreading by streaking in the usual manner over the entire plate. Frequently it was found necessary

TABLE 2

TRYPTONE-YEAST EXTRACT MEDIUM

	Per Cent
Tryptone (Bacto)	1.0
Yeast extract (Bacto)	0.2
Soluble starch (or glucose 0.2 per cent)	2.0
K_2HPO_4	0.5
Sodium chloride	0.5
Cysteine-hydrochloride	0.05
Redistilled H_2O	To volume

to streak once halfway down the middle lightly, then following this at right angles with a sterile needle to cover the entire plate. In most instances the desired colonies would be seen confluent with other micro-organisms on the more heavily streaked plates. When using inhibitory agents, such as dyes or antibiotics, the inoculum was increased to 0.05–0.1 ml. and spread over the entire plate with a sterile bent-glass rod or a straight inoculating needle.

Incubation of the streaked plates was carried out under anaerobic conditions in a vacuum desiccator jar at 37° C. The jar was exhausted of air and flushed with either carbon dioxide or nitrogen. An alkaline methylene blue tube was used as the anaerobic indicator. Sodium hydroxide and pyrogallic acid were employed in some of the earlier isolations, along with the flushing gas, but were later omitted.

Isolations of individual colonies of *L. buccalis* were accomplished with fine-bore Pasteur pipettes (0.25–0.5 mm. in diameter) after 2–3 days' incubation of the initial plates. This procedure was carried out under a dissecting microscope because the colony sizes were small and the contaminating colonies so numerous. With the limited period of incubation, we were able to restrict the growth of the undesirable colonies and achieve the desired isolation of the *L. buccalis*. Purification of the individual strains was accomplished by serial restreaking on agar plates. Longer periods of incubation were employed after initial isolation, to allow the colonies to grow larger and permit easier handling, usually 4 or 5 days. Maintenance of the individual isolates was

achieved by weekly inoculations into semisolid media, incubating under anaerobic conditions for 3 days, followed by storage at 4° C.

RESULTS

Isolation and media.—Isolation of the *L. buccalis* was most effective in the presence of soluble starch and the absence of glucose in either of the basal media described. This may have been due to an indirect effect of an unavailable energy source for most of the other organisms present. This was brought out by longer incubation of the initial plates and the resultant overgrowth by the other micro-organisms. Incubation periods of 2–3 days were used in the initial isolations to limit this overgrowth. The colonies were very tiny, but, with continued practice, we were able to isolate the filamentous forms from the others by using a very fine Pasteur pipette.

Alterations of the media by depletion or addition of different nutrients were tried, in order to increase the numbers and facilitate isolation of these forms, but with little success. Other authors[10, 13, 15] recommended dyes in different concentrations to inhibit the majority of the other forms. We therefore tried some dyes, such as crystal violet and brilliant green, as recommended by these authors and were successful in isolating strains of anaerobic filamentous micro-organisms. However, the dye color interfered with the ease of observation of these colonies and made isolation difficult. Antibiotics, such as sulfathiazole and bacitracin, were tried in varying concentrations with very little improvement. The possibility of producing variants by the use of these techniques influenced our decision to abandon the use of dyes and other inhibitory agents in favor of the more "natural" artificial environment commonly used in the laboratories for purposes of isolating micro-organisms.

Maintenance of these isolates was improved when soluble starch was replaced by glucose, and the glucose concentration was kept at a minimum of 0.1–0.2 per cent, with the buffering capacity adjusted to a molar ratio of approximately 1:3 or 1:4 of glucose to phosphate. Phosphate at 0.5 per cent concentration gave about the maximum efficiency that could be attained with good growth. Increasing the amount did not improve the growth, and concentrations between 1.0 and 2.0 per cent were slightly inhibitory. Glucose concentrations of 0.1 and 0.2 per cent in either brain-heart or tryptone media were sufficient to produce adequate growth for maintenance and storage of these cultures. Concentrations of 0.5–1.0 per cent improved growth considerably, but survival under 4° C. storage was poor, with cultures dying in a few days. Higher concentrations of 2.0–5.0 per cent were definitely inhibitory. Yeast extract in 0.2 per cent amounts increased the growth of these forms when other components were kept minimal.

Oxygen sensitivity.—Sensitivity to oxygen was detected in the early studies, as initial isolation plates incubated under aerobic conditions were not fruitful for isolation of this micro-organism. The various strains of *L. buccalis* isolated in our laboratory and strain No. 4 from Hamilton and Zahler[12] were grown in the presence and absence of carbon dioxide. Introduction of partial pressure of carbon dioxide produced better growth under anaerobic conditions, while a partial pressure of nitrogen in place of carbon dioxide allowed growth of these forms in far less amount than that seen with carbon dioxide. Five to 10 per cent carbon dioxide was found stimulatory for growth under the anaerobic conditions produced in the vacuum desiccator jars. Filling the

desiccator jars with carbon dioxide back to atmospheric pressure did not improve the growth.

Either a strict anaerobic condition or a microaerophilic condition was necessary to secure good growth on the surface of agar plates. In plates that were inoculated heavily and incubated aerobically, only the area of very heavy inoculation showed a scanty type of growth which, if transfered early and incubated anaerobically, would demonstrate the presence of viable organisms. Because of claims by many workers in this field that these micro-organisms adapted to "aerobic" growth after a few transfers, attempts were made to adapt several isolated strains in our laboratory. Successive transfers were made in deep, semisolid media with 0.2 per cent agar added to the basal media described earlier with the addition of 0.05 per cent cysteine hydrochloride. The resultant growth was very good, starting 1 cm. from the top and extending downward

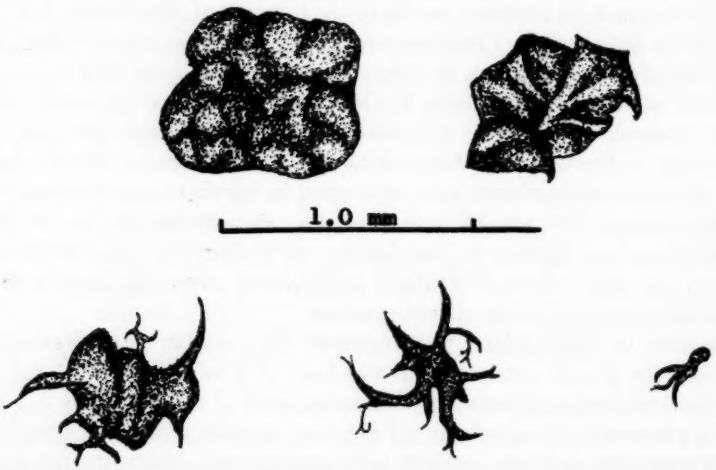

FIG. 1.—Drawings of a strain of *L. buccalis* which grew under strict aerobic conditions on the surface of brain-heart infusion agar medium supplemented with 10 per cent ascitic fluid. Colonial variants appeared on the same plate incubated for four days at 37° C. Microscopic morphology of individual cells and filaments was similar to those of the same strain grown under anaerobic conditions in a partial pressure of 10 per cent carbon dioxide.

along the inoculation stab. Transfers were made by taking the growth that appeared closest to the top as these tubes were allowed to incubate under aerobic conditions. Inoculum was deposited in fresh semisolid deep media with a Pasteur pipette spreading the micro-organisms equally at all depths to the extreme top. This procedure was followed through over 100 transfers made twice a week. At various intervals, attempts were made to grow them aerobically by streaking heavily on agar plates and testing at 37° C. We never obtained good growth on the surface of agar media incubated under these conditions during all these transfers. The type of growth that occasionally appeared was a thin, filmy one with scattered tiny colonies approximately 0.5–0.75 mm. in diameter. A few strains were able to show a very pleomorphic limited growth under aerobic conditions on an agar plate medium if supplemented with 10.0 per cent ascitic fluid. Figure 1 is a drawing illustrating different colonial types of one strain appearing on an agar plate supplemented with 10.0 per cent ascitic fluid and incubated aerobi-

cally. Microscopically, the individual cells did not differ from those grown under anaerobic conditions.

Morphology.—Pleomorphism is exhibited by this micro-organism both in colony morphology and in microscopic morphology. Figure 1 illustrates the variations in colony morphology exhibited by one strain on the same medium under the same conditions. The colonies have several variations which have been described by different workers, and they include the thatched, filamentous, streaming edges, and a "Medusa-head" appearance. The Medusa-head appearance results from longer (4–5 days) incubation, while the other forms can be seen with shorter (24–48 hours) incubation

FIG. 2.—Two typical *L. buccalis* colonies on the left with corresponding microscopic pictures of filaments and cells taken from tryptone-yeast extract agar plates incubated anaerobically for 24 hours at 37° C. Two intermediate types (variants) on the right under similar conditions.

periods. All these colonies have a characteristic soft consistency and differ in this respect from the facultative forms being studied by others.[16–19] The individual colonies are easily isolated from the surface of the agar plates by the use of ordinary inoculating needles or, as some workers have done, with fine-drawn Pasteur pipettes.

The variations in colonial forms appear to be due to the age of the individual colonies rather than to the inherent characteristics of this group. In general, if the cultures are allowed to incubate as long as 4 days, the majority of the colonies will have the Medusa-head appearance with occasional filamentous smaller colonies. Besides the time element, there is a factor of moisture content which can determine the degree of streaming and filamentation that appears on the agar surface. Figures 2 and 3 show

different colony types exhibited by different strains of *L. buccalis*. The Medusa colonies in Figure 2 were the type that we generally observed, and the corresponding microscopic pictures showed that the cells did not tend to form long filaments. The other two colony pictures in Figure 2 had a filamentous appearance, with a correspondingly longer filamentation occurring in the microscopic photographs. Figure 3 shows colonies of strains which might not be considered "true" *L. buccalis*, in that their fermentation patterns differed slightly from those we have classified as *L. buccalis*. These cultures showed a tendency to form more filamentation and thatched appearances rather than the usual Medusa colonies. The two strains on the right of Figure 3 pro-

FIG. 3.—Two variant colony morphologies by typical *L. buccalis* cultures with their corresponding microscopic pictures taken from 24-hour tryptone-yeast extract agar plates incubated anaerobically at 37° C. Two strains of gas-producing filamentous micro-organisms under similar conditions on the right.

duced a little gas when glucose was added as a carbon source. This characteristic was different from the other strains of *L. buccalis*.

The variability exhibited by the microscopic morphology of this micro-organism has been seen in the majority of the strains isolated to date. In general, they can vary in length from "short" rods (3–4 μ), which are rather scarce, to long filaments composed of several segments (40 μ or more) and have aptly been described as "logs," in contrast to rodlike micro-organisms by Bøe.[10] Bizarre irregular forms have been described by some workers, and these forms have been seen in our isolated strains as well —large coccoid bodies, bulbous swellings at the end or in the middle of a filament, and "ropelike" twisted forms composed of two separate elements twisted around each

other. Forms that appeared like ends of a dog bone, suggesting a possible beginning of branching, have been observed in wet-mount preparations. Other filaments with short protrusions from their sides with no indication of septation at the junction were also observed. These protrusions never exceeded the length of 1.5–2.0 μ, and attempts to observe them in microcolonies were unfruitful. All these forms appeared in cultures incubated for 24 hours or longer and more often in broth, though rarely in semisolid or agar plate media.

The bulbous swellings and granular appearance in many of the strains isolated suggested some stage in a life-cycle of the organisms, such as a resistive spore stage or a filterable phase. Attempts to demonstrate spores were unsuccessful. Heating cultures to 80° and 60° C. for periods of 5 and 10 minutes resulted in sterile suspensions. Staining techniques to demonstrate the existence of spores were equally negative. The extreme granulation which was observed as uniform neither in amount nor size was arranged in a random fashion in the filaments. These granules stained a yellow-orange with Grams iodine when the cells were cultured in the presence of glucose and the absence of starch. Cultures incubated in the presence of starch stained in the same manner exhibited a bluish color in the granules. Tests for a filterable stage in the life-cycle of these isolates were attempted. Heavily growing broth cultures incubated for 4 days were filtered through sterile sintered glass filters and asbestos Seitz filter pads. All filtrates gave negative results when tested for viability under anaerobic conditions normally used to grow these cultures.

Cells stained by the Gram technique gave variable results. Very young cells, 4–8 hours' incubation, gave definite Gram-positive reactions. Older cells, incubated 72 hours or more, gave variable results, many with a thin Gram-negative sheath surrounding large numbers of Gram-positive granules. Figures 4 and 5 are electron micrographs of chromium-shadowed whole-mount preparations of 24-hour cultures of *L. buccalis* showing the variations in length of the individual segments and how they are connected to form the long filaments.

Lyophilization and storage.—Cultures showing uniform colonial morphology were tested for viability after lyophilization and storage at room temperature. The cultures were grown in broth and, after 24 hours' incubation at 37° C. under anaerobic conditions, were centrifuged and washed and resuspended in about one-third the volume of sterile skimmed milk and lyophilized. The individual tubes were sealed under vacuum, with the final pressure being under 100 μ or less. The viability of each culture was tested after each step in order to evaluate the efficiency of the procedures on a qualitative basis. The lyophilized cultures have been tested periodically over the past several years, and they have maintained their viability. The oldest has been a little over 2 years. Some strains were also tested in the same manner, replacing the skimmed milk with sheep serum. These gave equally good results.

Viability under refrigeration storage at 4° C. was dependent on the conditions of growth in obtaining the cells. Ordinary growth conditions, where good growth was attained within 24 hours, as in the presence of 1 per cent glucose, and the buffering capacity of the medium was low, these micro-organisms produced an extreme acidity which was detrimental for their survival. This extreme acid production was avoided by reducing the glucose concentration to a minimum of 0.1–0.2 per cent and increasing the phosphate on a molar basis to 4:1 with respect to glucose. These micro-organisms

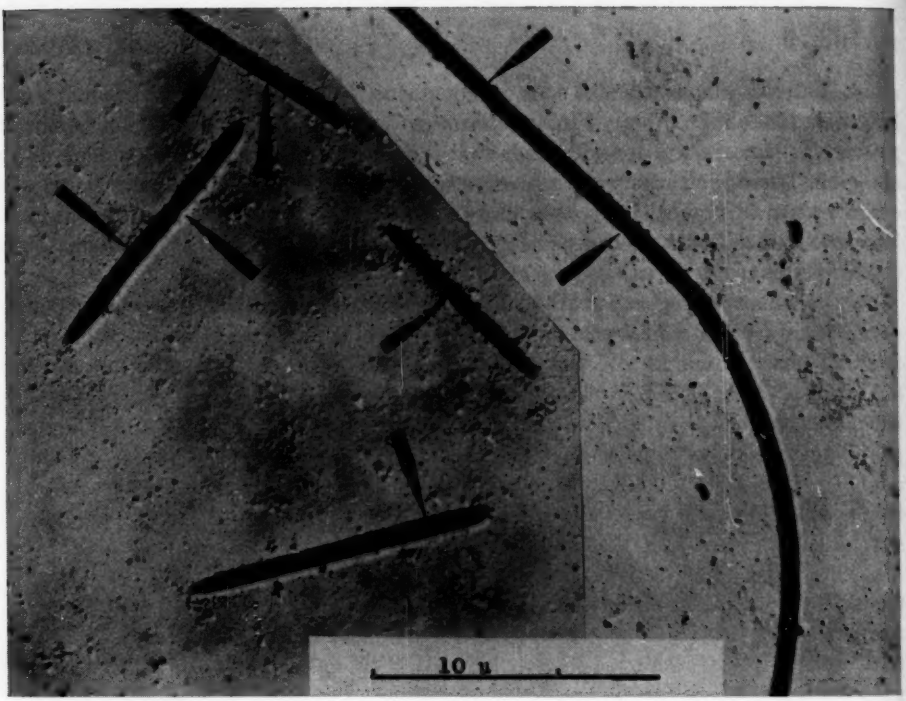

FIG. 4

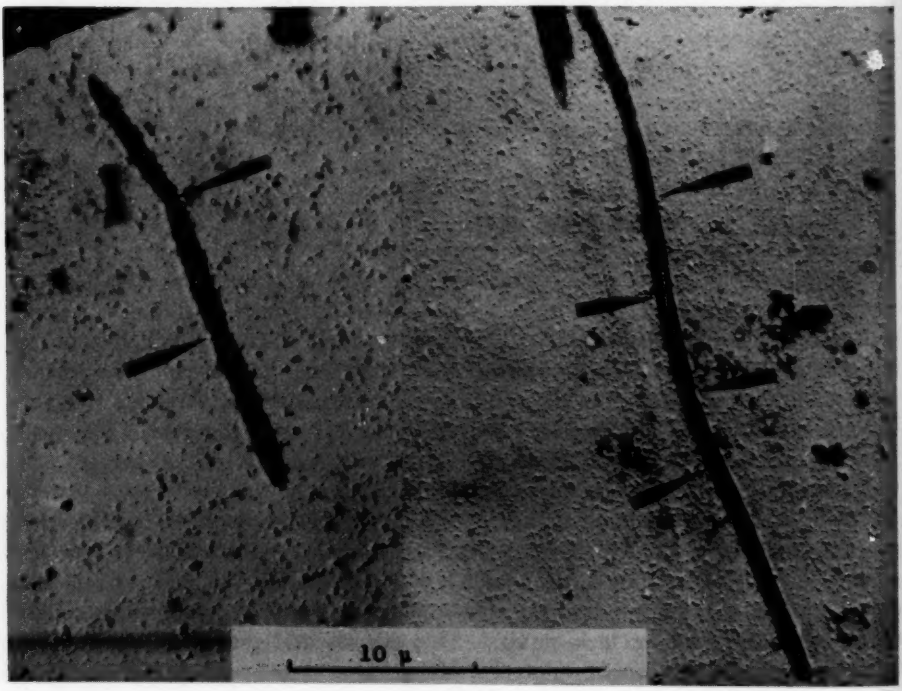

FIG. 5

FIGS. 4 and 5.—Chromium-shadowed electron micrograph preparations of *L. buccalis* showing constrictions along the cell wall of filaments indicating septation. Uneven granulation is also apparent, as is seen in many wet preparations viewed in the phase-contrast microscope. Variation in length of the individual elements is striking.

could survive a month or more in storage if allowed to grow under these specified conditions in a semisolid medium for from 48 to 72 hours' incubation followed by refrigeration at 4° C. Storage at —70° C. of washed cells harvested from 24-hour broth cultures and resuspended in medium of the same composition before being frozen was also successful for longer than 6 months. Similarly treated cells stored at —20° C. were not viable after 6 months.

DISCUSSION

The problem in initial isolation of *L. buccalis* is to allow these forms to grow while inhibiting growth of other forms that are normally found in the mouth. Because there are other micro-organisms in the oral cavity which grow at a very rapid rate, they obscure the colonies of the filamentous micro-organisms, which require longer periods of incubation in initial isolations on agar plates. One can find these filamentous colonies protruding out from under a heavy streak, giving the impression that the adjoining colonies are elaborating some substance(s) beneficial for their growth.

Modification of the medium by replacing the glucose with soluble starch aided in the better isolation of *L. buccalis* and, at the same time, inhibited the growth of the other forms. We washed the soluble starch, during our first attempts, three times in four volumes of redistilled water to remove any water-soluble components but later found that unwashed preparations gave good results. Allowing these plates to incubate for long periods permitted the other forms to grow and obscure the desired filamentous forms. Thus the agar plates were examined after a relatively short incubation period.

The carbon dioxide requirements were shown by the decrease in growth obtained when the partial pressure of carbon dioxide was replaced by nitrogen. In the presence of sodium hydroxide and pyrogallic acid mixtures to obtain anaerobic conditions, carbon dioxide was seen to stimulate growth also.

Morphologic studies on *L. buccalis* indicate that this organism is pleomorphic with regard to its microscopic picture and also that different strains can exhibit variations in colonial morphology but that any one strain is relatively stable. Aerobic cultures grown in the presence of 10 per cent ascitic fluid do exhibit some pleomorphism in their colonial morpohologies. Microscopic variations are largely those of filament lengths, which can range from a few to several hundred microns. The elements very rarely appear singly but mostly in pairs or several in chains with one end tapered and the other rounded to bulbous. Branching is suggestive but has not been definitely proved. Granulation is rather prominent in older preparations, and motility is absent. Cells themselves stain rather unevenly with the Gram technique, but the younger cells are definitely Gram-positive. The dominant colony morphology appears to be the Medusa-head type, which arises from the thatched-appearing colonies. This is perhaps because they stand out more and are easier to isolate. The streaming-edge type and filamentous type of colony were usually so obscured by other organisms that it was difficult to isolate them. There is also the matt type of colony, which appears to be the result of further growth by the filamentous type because of its round appearance and fringe type of border. Because of the colonial morphology of these forms and the similarity with other filamentous varieties, it is easy to see how confusion between the different species can arise.

Data indicate that this organism is distinct from other filamentous forms. The

genus and species name *L. buccalis* should be reserved for this particular micro-organism,[20] and it should not be confused with other filamentous varieties, as in the past. After reviewing the past literature and the earliest use of the term, there is no doubt that the earlier workers[2–4] were referring to this particular organism. The other filamentous varieties which have been given this name are, in reality, some other species and should be so classified. Further work on their biochemical and physiological characteristics, as well as a possible study on their serological reactions, should resolve the problem of their classification.

We have failed to isolate similar micro-organisms from other sources, such as fermenting processed vegetables, soil, etc. These organisms appear to be limited to the oral cavity and are observed there in large numbers, regardless of the pathologic conditions.

SUMMARY

A morphologic study of *L. buccalis* has been presented with a comparison with other studies by previous investigators. A method of maintaining these cultures either by lyophilization or by refrigerator storage was successfully achieved and has been presented.

There is a certain degree of pleomorphism exhibited by these cultures. However, in general, the morphology, with regard to both macroscopic or colonial and microscopic appearances, of this species appears to be quite stable. Further studies regarding their biochemical and other physiologic activities should be done to differentiate this species from other oral filamentous micro-organisms or filamentous micro-organisms from other sources.

The species *L. buccalis* appears to have a definite requirement for carbon dioxide and is incapable of reproduction at the same rate or magnitude in the absence of carbon dioxide either under anaerobic or microaerophilic conditions. Aerobic conditions require a lowering of Eh potential, as shown by the presence of good growth under these conditions when either cysteine or thioglycollate is added to the medium.

The classification and differentiation of this species has been briefly discussed, with the recommendation of retaining the genus and species name for this micro-organism.

REFERENCES

1. Breed, R. S., Murray, E. G. D., and Smith, N. R. *Bergey's Manual of Determinative Bacteriology*. 7th ed. Baltimore: William & Wilkins Co., 1957.
2. Buehlmann, F. Ueber eine eigenthümliche, auf den Zähnen des Menschen vorkommende Substanz, *Arch. Anat., Physiol. wiss. Med.*, p. 442, 1840.
3. Robin, C. *Histoire naturelle des végétaux parasites qui croissent sur l'homme et sur les animaux vivants*, p. 345. Paris: J. B. Baillière, 1853.
4. Trevisan, S. C. V. Letture della classe di scienze matematiche e naturali, *Reale Ist. Lombardo Sci. et Lettre*, Series II, **4**:133, 1879.
5. Miller, W. D. *Micro-organisms of the Human Mouth*. Philadelphia: S. S. White Dental Mfg. Co., 1890.
6. Kligler, I. J. A Biochemical Study and Differentiation of Oral Bacteria, with Special Reference to Dental Caries, *J. Allied D. Soc.*, **10**:141, 1915.
7. ———. Biochemical Studies of the Relations of Oral Microorganisms to Dental Caries, *ibid.*, p. 282.
8. Wherry, W. B., and Oliver, W. W. *Leptothrix innominata* (Miller), *J. Infectious Diseases*, **19**: 299, 1916.
9. Thjøtta, T., Hartmann, O., and Bøe, J. A Study of the *Leptotrichia* Trevisan, *Norske Videnkaps-Akad. Oslo, I, Math.-nat. Kl.*, No. 5, p. 5, 1939.

10. BØE, J. *Fusobacterium*. Studies on Its Bacteriology, Serology, and Pathogenicity, *Norske Videnkaps-Akad. Oslo, I, Math.-nat. Kl.*, No. 9, p. 9, 1941.
11. JACKINS, H. C., and BARKER, H. A. Fermentative Processes of the Fusiform Bacteria, *J. Bacteriol.*, **61**:101, 1951.
12. HAMILTON, R. D., and ZAHLER, S. A. A Study of *Leptotrichia buccalis*, *J. Bacteriol.*, **73**:386, 1957.
13. BAIRD-PARKER, A. C., and DAVIS, G. H. G. The Morphology of *Leptotrichia* Species, *J. Gen. Microbiol.*, **19**:446, 1958.
14. KASAI, G. J. A Study of Mediums for the Growth and Isolation of Filamentous Mouth Organisms, *J. Infectious Diseases*, **96**:279, 1955.
15. BAIRD-PARKER, A. C. Isolation of *Leptotrichia buccalis* and *Fusobacterium* Species from Oral Material, *Nature*, **180**:1056, 1957.
16. BULLEID, A. An Experimental Study of *Leptothrix buccalis*, *Brit. D. J.*, **46**:289, 1925.
17. BIBBY, B. G., and BERRY, G. P. A Cultural Study of Filamentous Bacteria Obtained from the Human Mouth, *J. Bacteriol.*, **38**:263, 1939.
18. GILMOUR, M. N., and HUNTER, P. The Growth Characteristics of an Oral Gram-Positive Filamentous Organism, *J. D. Res.*, **37**:10, 1958.
19. RICHARDSON, R. L., and SCHMIDT, J. An Oral Filamentous Micro-organism: Cultural Characteristics and Microbial Relationships Affecting Growth, *J. D. Res.*, **38**:1016, 1959.
20. JUDICIAL COMMISSION OPINION. Status of Generic Names of Bacteria Proposed by Trevisan, *Intern. Bull. Bacteriol., Nomenclature and Taxonomy*, **3**:96, 151, 1953; and **4**:152, 1954.

Variations in the Morphology of Mandibular First Molars of Albino Rats

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Studies of dental caries in man and in rats suggest a relation between this disease and the shape and size of certain teeth. Hyatt,[1] reviewing the literature prior to 1930, was convinced that a cause-and-effect relationship existed between pits, fissures, and dental caries. This opinion was supported by Bossert,[2, 3] who concluded that "the steeper the sides of the valley the greater the likelihood of caries in the central pit" of permanent maxillary first molars and deciduous maxillary second molars. The experimental evidence of greater mean widths of the occlusal grooves in mandibular molars of 40-day-old caries-susceptible rats, presented by Kifer, Hunt, Hoppert, and Witkop,[4] contradicts the observations of Hyatt and Bossert. However, no attempt was made by Kifer *et al.* to determine whether tooth wear was responsible for the observed differences. Furthermore, no differences were noted between mean fissure widths in molars on the right and left side, although more caries occurred on the right side.

Grainger, Paynter, and Shaw[5] explored the association between the gross morphology of maxillary first molars and the predisposition to dental caries in 12–14-day-old rats, raised from either caries-resistant or caries-susceptible strains. The teeth had probably not been exposed to wear, and, in all likelihood, dental caries had not developed. It was observed that the average mesiodistal crown diameter, especially at the cervix, and the mesio-occlusal fissures were both narrower in the teeth from caries-susceptible rats.

The present study was undertaken to establish the normal range of variation in the size and shape of the mandibular first molars of albino rats. Modifications resulting from attrition were also determined. The findings of this investigation are fundamental to future studies of factors that influence the shape, as well as the external and internal dimensions, of molar crowns and also the relation of experimentally induced dental caries to tooth morphology.

MATERIALS AND METHODS

A total of 108 male and the same number of female albino rats, descendants of the Sprague-Dawley strain, were used for this study. In order to provide an opportunity for comparable nutrition, each litter was reduced to 8 pups by the breeder* imme-

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* The Holtzman Co., Madison, Wisconsin.

diately after birth. All rats were weaned at 21 days of age. Purina rat laboratory chow and tap water were availble ad libitum throughout the experimental method.

Twelve rats of each sex were killed when 41 days old, 20 males and 20 females were killed at age 61 days, and the remaining 152 rats were killed at the age of 91 days. Consequently, teeth were obtained with varying degrees of occlusal wear.

Comparable mid-coronal ground sections, 95–105 μ thick, were prepared according to a reproducible imbedding and grinding procedure developed for this study. A row of 8–10 mandibular first molars of similar buccolingual crown size were aligned in specially constructed steel matrices. Previous dehydration of the molars in absolute alcohol insured bonding with imbedding material.* The epoxy resin cured at room temperature and exhibited little dimensional change ($<$ 2 per cent).

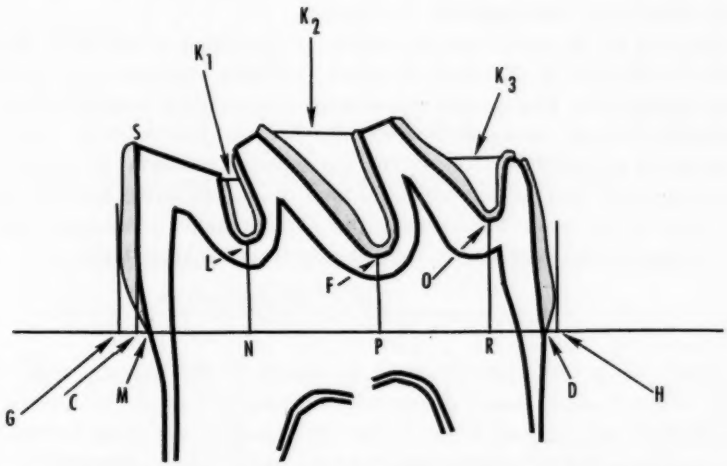

FIG. 1.—Tracing of the mid-coronal section of the mandibular first molar of an albino rat showing landmarks and lines used for measuring external and internal tooth dimensions.

Mid-coronal sections of the molars were obtained by removing equal amounts from the buccal and lingual surfaces. Precise grinding was facilitated by controlled horizontal and vertical feeding of the sections to a revolving diamond wheel. The vertical feed was regulated by means of a micrometer advance mechanism that permitted removal of predetermined amounts, as small as 3–4 μ. The fine grit of the diamond wheel made polishing of the specimens unnecessary. Upon completion of the grinding and clearing in Xylene,† the sections were mounted in Permount.‡

Tracings were made of the external enamel contour, the dentino-enamel border, and the pulpo-dentinal surface of each tooth (Fig. 1) with a microprojector§ at standardized magnification ($\times$46.2). On the tracings, the cervical mesial (M) and distal (D) dentino-enamel junctions were located. A straight line (M–D) was drawn through these points, denoting the cervical mesiodistal crown dimension. Further-

*Hysol #6020. Houghton Laboratories Inc., Olean, N.Y.

† Merck and Co. Inc., Rahway, N.J.

‡ Fisher Scientific Co., Fairlawn, N.J.

§ Bausch & Lomb Tri-Simplex.

more, a perpendicular was drawn to the extended *M–D* line from the most mesial and distal aspects of the proximal enamel surfaces, giving points *G* and *H*, respectively. The line *G–H* represented the maximal mesiodistal crown size.

In the base of the mesial, central, and distal occlusal grooves, the most apical points, *L*, *F*, and *O*, respectively, were located on the dentino-enamel border. Through these points the perpendiculars *N–L*, *F–P*, and *O–R* were constructed to the *M–D* line to represent the mesial, central, and distal internal crown heights.

In order to determine the effects of tooth wear, four additional measurements were taken. The mesial external crown height (*S–C*) was represented by a perpendicular from the occlusal dentino-enamel junction (*S*) of the mesial cusp to the extended *M–D* line. The width of the mesial, central, and distal occlusal grooves (K_1, K_2, and K_3, respectively) was measured parallel to the *M–D* line at the dentino-enamel junction of the most worn cusp for each groove.

The location of all points and dimensions is illustrated in Figure 1, in which the thickness of the lines in the original tracing has been accentuated to permit photographic reproduction. The measurements were made with a vernier caliper.*

Measurements and tracing techniques were both subject to error. Two procedures were employed to calculate these errors. On separate occasions a tracing was made for the same tooth, and on each tracing a total of 23 dimensions was measured twice. On the basis of the mean differences of the 23 dimensions in tracing 1 and those in tracing 2, the tracing error was calculated according to the formula

$$\text{S. D. error} = \left(\frac{\text{Sum of differences}^2}{2n}\right)^{1/2}.$$

Measurement error was also calculated by means of the formula, from differences between 10 independent measurements of 23 dimensions on one of the tracings. The results of these calculations showed that the standard deviation for the errors of tracing and the errors of measurement were 1.2 and 0.5 mm., respectively.

RESULTS AND DISCUSSION

The data were studied separately for each sex. The findings, converted to actual dimensions, are presented for tooth dimensions unaffected by occlusal wear (Table 1) and for dimensions that were altered by tooth wear (Table 2). The mean maximal mesiodistal crown size (*G–H*) was greater in molars of male than of those of female rats, namely, 3.08 and 3.05 mm., respectively. This difference was statistically highly significant ($P < 0.001$). Because the cervical mesiodistal crown dimension (*M–D*) was very similar in both sexes, it is suggested that a greater proximal bulging of the molar tooth in males was responsible for the difference in the maximal mesiodistal crown size between the sexes.

The mean buccolingual crown size was 1.87 mm. in molars of males and 1.84 mm. in females. This difference was not statistically significant because of the large individual variation expressed by the standard deviations.

Internal mesial, central, and distal crown heights were similar in the mandibular first molars of both sexes and averaged 0.57, 0.52, and 0.75 mm., respectively. Relative variability, as expressed by the coefficient of variation, was largest (C.V. = 10.5)

* L. S. Starrett Co., Athol, Mass.

TABLE 1*

CROWN DIMENSIONS, UNAFFECTED BY OCCLUSAL WEAR, OF MANDIBULAR FIRST MOLARS OF ALBINO RATS

Area	Sex	Mean (Mm.)	S.E.$_M$ (Mm.)	S.D. (Mm.)	C.V. (Per Cent)	Range (Mm.)	No.
Buccolingual size	♂	1.87	0.015	0.14	7.4	1.74–1.97	91
	♀	1.84	0.022	0.20	10.2	1.69–1.95	80
Maximal mesiodistal size (*G–H*)	♂	3.08	0.007	0.08	2.6	2.87–3.24	106
	♀	3.05	0.007	0.07	2.3	2.87–3.20	82
Mesiodistal cervical size (*M–D*)	♂	2.79	0.017	0.18	6.5	2.61–3.03	106
	♀	2.78	0.007	0.08	2.9	2.58–2.94	83
Internal mesial height (*L–N*)	♂	0.57	0.005	0.06	10.5	0.43–0.76	106
	♀	0.57	0.005	0.06	10.5	0.42–0.69	84
Internal central height (*F–P*)	♂	0.52	0.003	0.04	7.7	0.44–0.63	106
	♀	0.51	0.003	0.04	7.8	0.40–0.59	84
Internal distal height (*O–R*)	♂	0.75	0.003	0.04	5.3	0.64–0.83	106
	♀	0.74	0.003	0.04	5.4	0.64–0.81	84

*S.E.$_M$ = standard error of the mean; S.D. = standard deviation; C.V. = coefficient of variation.

TABLE 2

CROWN DIMENSIONS, AFFECTED BY OCCLUSAL WEAR, OF MANDIBULAR FIRRT MOLARS OF ALBINO RATS

Area	Age (Days)	Sex	Mean (Mm.)	S.E.$_M$ (Mm.)	S.D. (Mm.)	C.V. (Per Cent)	Range (Mm.)	No.
Mesial external height (*S–C*)	41	♂	1.11	0.020	0.09	8.1	0.91–1.31	21
	41	♀	1.14	0.019	0.09	7.9	0.91–1.30	23
	61	♂	1.05	0.017	0.07	6.6	0.93–1.14	17
	61	♀	1.01	0.022	0.09	8.9	0.89–1.14	17
	91	♂	0.98	0.011	0.09	9.2	0.72–1.16	66
	91	♀	1.01	0.025	0.17	16.8	0.87–1.14	44
Mesial occlusal groove (K_1)	41	♂	0.08	0.004	0.02	22.5	0.04–0.11	21
	41	♀	0.07	0.004	0.02	28.8	0.04–0.10	23
	61	♂	0.11	0.007	0.03	27.3	0.05–0.17	19
	61	♀	0.10	0.004	0.02	20.0	0.06–0.14	18
	91	♂	0.12	0.003	0.03	25.0	0.06–0.19	66
	91	♀	0.11	0.004	0.03	18.2	0.05–0.17	43
Central occlusal groove (K_2)	41	♂	0.49	0.013	0.06	12.2	0.39–0.61	21
	41	♀	0.48	0.014	0.07	14.6	0.30–0.55	23
	61	♂	0.44	0.016	0.07	15.9	0.32–0.56	19
	61	♀	0.44	0.016	0.07	15.9	0.38–0.57	18
	91	♂	0.34	0.004	0.04	11.8	0.26–0.44	65
	91	♀	0.34	0.010	0.05	20.6	0.24–0.57	43
Distal occlusal groove (K_3)	41	♂	0.39	0.011	0.05	12.8	0.28–0.47	21
	41	♀	0.37	0.010	0.05	13.5	0.26–0.49	23
	61	♂	0.27	0.012	0.05	18.5	0.21–0.41	18
	61	♀	0.25	0.012	0.05	20.0	0.18–0.31	17
	91	♂	0.20	0.004	0.04	20.0	0.13–0.29	66
	91	♀	0.21	0.006	0.04	19.0	0.16–0.30	43

for the mesial internal crown height and smallest (C.V. = 5.3) for the distal internal crown height. However, one cannot infer from these coefficients of variation that the mesial height was twice as variable as the distal height because the coefficients were influenced by the mean mesial and distal internal crown heights, which differed markedly (0.18 mm.).

Correlation coefficients were calculated to determine the relation between the maximal mesiodistal and buccolingual crown size, the maximal mesiodistal dimension and distal internal height, and between the distal and central, distal, and mesial, and central and mesial internal crown heights (Table 3). The statistical significance of the correlation coefficients was determined by the ratio $r/\text{S.E.}_r$.

The correlation between maximal mesiodistal and buccolingual crown size was relatively high ($r = +0.781$ and $+0.840$) and statistically highly significant. Mod-

TABLE 3

CORRELATION COEFFICIENTS FOR CROWN DIMENSIONS, UNAFFECTED BY OCCLUSAL WEAR, OF MANDIBULAR FIRST MOLARS OF ALBINO RATS

Areas	Sex	$r \pm \text{S.E.}_r$	$r/\text{S.E.}_r$	No.
Maximal mesiodistal size (*G–H*) and buccolingual size	♂	+0.781±0.042	18.6	85
	♀	+0.840±0.049	16.9	35
Maximal mesiodistal size (*G–H*) and distal internal height (*O–R*)	♂	+0.043*	.....	106
	♀	+0.124*	.....	82
Distal internal height (*O–R*) and central internal height (*F–P*)	♂	+0.598±0.062	9.7	106
	♀	+0.333±0.096	3.5	84
Distal internal height (*O–R*) and mesial internal height (*L–N*)	♂	+0.421±0.080	5.3	106
	♀	+0.470±0.085	5.5	84
Mesial internal height (*L–N*) and central internal height (*F–P*)	♂	+0.642±0.057	11.3	106
	♀	+0.582±0.072	8.1	84

* Not statistically significant.

erate correlation, but also statistically significant, was observed between the distal and central, distal and mesial, as well as mesial and central internal crown heights. The correlation ($r = +0.043$ for males and $+0.124$ for females) between maximal mesiodistal size and distal crown height was not statistically significant. This lack of association between vertical and horizontal tooth dimensions had already been expressed by the fact that there were significant sex differences in the maximal mesiodistal size but not in any of the three internal crown heights. The findings suggested that the vertical and horizontal directions of tooth formation were controlled independently.

The mandibular first molar crowns were fully formed when rats were only about 10 days old. The sex difference in tooth size was therefore established much earlier than sex differences in craniofacial skeleton. The craniofacial dimensions in male rats did not exceed those of the females until the animals were about 2 months of age, according to Massler and Schour.[6] A difference in the timing of attaining tooth size and skeletal size was also observed in humans and may in part be responsible for

the lack of relation between tooth size and dental arch size that was observed in instances of crowding and spacing.[7]

The dimensions affected by occlusal wear are presented in Table 2. From the shape of the occlusal grooves, shown in Figure 1, it was expected that the occlusal opening (K_1) of the mesial groove would increase as a result of wear and that the occlusal opening of the central (K_2) and distal (K_3) grooves would decrease. Furthermore, the external mesial crown height (S–C) could be expected to decrease also.

In spite of the large individual differences, certain statistically significant dimensional changes could be defined from the data of this cross-sectional study. The occlusal opening of the mesial groove became wider (0.03 mm.) between 41 and 61 days (C.R. = 3.7 for males and 5.3 for females), and it continued to increase in width to 91 days at a much lower rate (0.01 mm.). The occlusal aspects of the central groove, however, decreased only about 10 per cent in its width between 41 and 61 days, and markedly (0.10 mm.) from 61 to 91 days (C.R. = 5.3 for males and 6.1 for females).

The occlusal width of the distal groove decreased substantially (0.12 mm.) in both sexes between 41 and 61 days (C.R. = 7.4 for males and 7.7 for females). During the period from 61 to 91 days the width of the distal groove decreased more in males (0.07 mm.) than in females (0.04 mm.), the respective critical ratios being 5.6 and 2.9. The external crown height decreased 0.13 mm. in both males and females between 41 and 91 days.

SUMMARY

A study of mandibular first molars of 216 albino rats showed that the maximal mesiodistal crown size was larger in male than in female rats. The mesiodistal dimension at the cervical level was similar for the molars of both sexes; therefore, it is suggested that the molars of male rats had a greater bulging of the proximal surfaces of their crowns. No sex differences in the mesial, central, and distal internal crown heights were observed.

The crowns of mandibular first molars were fully formed when the rats were about 10 days of age. The sex difference expressed in the maximal mesiodistal tooth size was thus established much earlier than sex differences in the craniofacial skeleton, which did not become apparent until the animals were about 2 months old.[6]

The maximal mesiodistal and buccolingual crown size was highly correlated. Moderate, but statistically significant, correlation was observed between the mesial and distal, central and distal, and mesial and central internal crown heights.

As a result of tooth wear, a statistically significant widening of the occlusal opening of the mesial groove and a narrowing of the distal groove took place between 41 and 61 days of age. The latter dimension continued to decrease at a rapid rate from 61 to 91 days, and the occlusal aspect of the central groove also became considerably narrower during this period. There was a decrease of 0.13 mm. in external mesial crown height in both males and females between 41 and 91 days.

The strain of rats used in this investigation has been inbred for many generations, and the animals had an opportunity for comparable nutrition during and after tooth development. Although food debris was found in all occlusal grooves of the molars, no dental caries lesions were observed clinically or microscopically.

The wide range of normal variation in the size and shape of rat molars, the early age when the crown morphology was established, as well as the marked occlusal changes due to wear, must be considered when studying the effects of nutritional, endocrine, and other factors on tooth morphology and the possible relation between the size and health of teeth.

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REFERENCES

1. Hyatt, T. P. A Review of the Literature on Pits and Fissures, *J. D. Res.*, **10**:659, 1930.
2. Bossert, W. A. The Relation between the Shape of the Occlusal Surfaces of Molars and the Prevalence of Decay, *J. D. Res.*, **13**:125, 1933.
3. ———. The Relation between the Shape of the Occlusal Surfaces of Molars and the Prevalence of Decay. II, *ibid.*, **16**:63, 1937.
4. Kifer, P. E., Hunt, H. R., Hoppert, C. A., and Witkop, C. J. A Comparison between the Widths of the Fissures of the Lower Molars of Caries-resistant and Caries-susceptible Albino Rats, *J. D. Res.*, **35**:620, 1956.
5. Grainger, R. M., Paynter, K. J., and Shaw, J. H. Differences in the Morphology and Size of the Teeth of a Caries-susceptible and a Caries-resistant Strain of Rats, *J. D. Res.*, **38**:105, 1959.
6. Massler, M., and Schour, I. The Growth Pattern of the Cranial Vault in the Albino Rats as Measured by Vital Staining with Alizarin Red S, *Anat. Rec.*, **110**:83, 1951.
7. Moorrees, C. F. A., and Reed, R. B. Biometrics of Crowding and Spacing of the Teeth in the Mandible, *Am. J. Phys. Anthropol.*, **12**:77, 1954.

The Effectiveness of Selection in Producing Laboratory Stocks Genetically Uniform for Resistance to Dental Caries

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Selection of breeders to produce a specified homozygous stock may be simple or very complicated. One can very easily secure a strain of rodents homozygous for a certain coat color, for complications are not numerous. But producing homozygosity for some other traits can be difficult. Several possible reasons for this may be stated briefly.

If some of the genes concerned are dominant, undesirable alleles cannot be detected by inspecting the animal. Our experience indicates that more than one pair of similar genes (multiple factors) are involved in resistance to dental caries in rats. The larger the number of such genes, the slower will be the progress toward homozygosity. The effect of some cariogenic genes might be nullified by other genes which are not their alleles (epistasis). "Modifying" genes might weaken or intensify the effects of the cariogenic genes. Some genes do not always produce a visible result, even when homozygous (low penetrance), and others do not have a marked effect (low expressivity).

The genes for dental caries, cariogenic oral micro-organisms, and diet are interacting factors in causing tooth decay. Dental caries in the rat is affected by so many circumstances that, in the selection of a resistant breeder, the caries record of its brothers, sisters, and parents, as well as its own, should be considered. High resistance of an animal in a sibship of lower average resistance may be due partly to some unknown non-genetic factor.

Such complications do not prevent selection against undesired genes, but they lower the rate at which the frequencies of such genes can be reduced. The consequences of such differences in the intensity of selection are the subject of this study.

We are making the conventional assumption that the initial population is in genetic equilibrium. Two types of formulas are derived: one for the rates of decline in the frequencies of the genes selected against and one for the increase in the desired homozygotes. Our experience suggests that the number of pairs of genes affecting dental caries is small and that there is no dominance within the pairs of alleles. We are arbitrarily using two pairs of genes, (A,a) and (B,b), in our demonstration. Many other models could be used, each with more than two pairs of genes, and within each model there could be many gene frequencies and rates of selection, but they would all yield results similar to the ones we present.

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Let p_1 = frequency of gene A; q_1 = frequency of gene a; p_2 = frequency of gene B; and q_2 = frequency of gene b.

For the pair (A,a) the frequencies of the genotypes will be $(p_1 + q_1)^2 = p_1^2 + 2p_1q_1 + q_1^2$, in which $p_1^2 = f$ AA, $2p_1q_1 = f$ Aa, and $q_1^2 = f$ aa. Similarly, for the pair of genes (B,b) the frequencies of genotypes will be $p_2^2 = f$ BB, $2p_2q_2 = f$ Bb, and $q_2^2 = f$ bb. The product $(p_1^2 + 2p_1q_1 + q_1^2)(p_2^2 + 2p_2q_2 + q_2^2)$ gives the genotypic frequencies involving both pairs of genes. The assumed degree of resistance to dental caries is indicated for each genotype in the accompanying table. The ideal objective is to eliminate genes a and b and to secure a population of AABB genotypes only.

Genotype	Frequency	Degree of Resistance to Caries (Phenotype)	Genotype	Frequency	Degree of Resistance to Caries (Phenotype)
AABB.....	$p_1^2p_2^2$	Very resistant	aaBB.....	$q_1^2p_2^2$	Intermediate
AABb.....	$2p_1^2p_2q_2$	Resistant	Aabb.....	$2p_1q_1q_2^2$	Susceptible
AaBB.....	$2p_1q_1p_2^2$	Resistant	aaBb.....	$2q_1^2p_2q_2$	Susceptible
AAbb.....	$p_1^2q_2^2$	Intermediate	aabb.....	$q_1^2q_2^2$	Very susceptible
AaBb.....	$4p_1q_1p_2q_2$	Intermediate			

It is assumed that the intermediate, susceptible, and very susceptible individuals can be identified and that all such genotypes are discarded in every generation. Selection against genes a and b is thus very intense at the beginning. It is assumed, also, that it is more or less difficult to distinguish AABb and AaBB from AABB by their phenotypic expressions, because of imperfect detection of carious cavities, slight variations in the ration (supposed to be constant), chance fluctuations in oral flora, etc. As a result, sometimes AABb and AaBB are unintentionally selected for breeding. All AABB individuals are bred. The proportion of AABb and AaBB animals detected and discarded is represented by s, so that $1-s$ is the fraction of these types erroneously retained as breeders.

To begin the analysis, what are the proportions of the various types of breeders after the undesirable types are discarded? Let N be the total number of individuals in the population before any animals are eliminated. Then

$$p_1^2p_2^2N = \text{the number of AABB breeders},$$

$$2p_1^2p_2q_2N(1-s) = \text{the number of AABb breeders},$$

$$2p_1q_1p_2^2N(1-s) = \text{the number of AaBB breeders}.$$

The total number of breeders is the sum of these three terms:

$$[(p_1^2p_2^2) + 2p_1^2p_2q_2(1-s) + 2p_1q_1p_2^2(1-s)]N$$

$$= [(p_1p_2)^2 + 2(1-s)(p_1p_2)(p_1q_2 + p_2q_1)]N$$

$$\text{Let } (p_1p_2)^2 + 2(1-s)(p_1p_2)(p_1q_2 + p_2q_1) = S.$$

Consequently,

$$\frac{(p_1p_2)^2N}{SN} = \frac{(p_1p_2)^2}{S} = \text{the frequency of the AABB breeders};$$

$$\frac{2p_1^2p_2q_2N(1-s)}{SN} = \frac{2(1-s)(p_1p_2)(p_1q_2)}{S} = \text{the frequency of the AABb breeders;}$$

$$\frac{2p_1q_1p_2^2N(1-s)}{SN} = \frac{2(1-s)(p_1p_2)(p_2q_1)}{S} = \text{the frequency of the AaBB breeders.}$$

The conventional "checkerboard" of elementary genetics can now be used to calculate the frequencies of the various matings among the above breeders. These frequencies are as follows:

$$\frac{(p_1p_2)^4}{S^2}, \quad \text{AABB} \times \text{AABB};$$

$$\frac{4(1-s)(p_1p_2)^3(p_1q_2)}{S^2}, \quad \text{AABB} \times \text{AABb};$$

$$\frac{4(1-s)(p_1p_2)^3(p_2q_1)}{S^2}, \quad \text{AABB} \times \text{AaBB};$$

$$\frac{4(1-s)^2(p_1p_2)^2(p_1q_2)^2}{S^2}, \quad \text{AABb} \times \text{AABb};$$

$$\frac{8(1-s)^2(p_1p_2)^2(p_1q_2)(p_2q_1)}{S^2}, \quad \text{AABb} \times \text{AaBB};$$

$$\frac{4(1-s)^2(p_1p_2)^2(p_2q_1)^2}{S^2}, \quad \text{AaBB} \times \text{AaBB}.$$

The genotypes among the total progeny from the above matings and the proportions of the various genotypes can easily be calculated. For example, the proportions of progeny from the mating AABb × AABb will be ¼ AABB + ½ AABb + ¼ AAbb, so that the frequencies of these offspring in the whole generation of progeny will be

$$\frac{(1-s)^2(p_1p_2)^2(p_1q_2)^2}{S^2}\text{AABB} + \frac{2(1-s)^2(p_1p_2)^2(p_1q_2)^2}{S^2}\text{AABb} + \frac{(1-s)^2(p_1p_2)^2(p_1q_2)^2}{S^2}\text{AAbb}.$$

The frequency of AABB among the offspring will be, after the terms are calculated,

$$\frac{(p_1p_2)^2[(p_1p_2)+(1-s)(p_1q_2+p_2q_1)]^2}{S^2} = \frac{(p_1p_2)^2[(p_1p_2)+(1-s)(p_1q_2+p_2q_1)]^2}{[(p_1p_2)^2+2(1-s)(p_1p_2)(p_1q_2+p_2q_1)]^2} = \frac{[(p_1p_2)+(1-s)(p_1q_2+p_2q_1)]^2}{[(p_1p_2)+2(1-s)(p_1q_2+p_2q_1)]^2}.$$

If we let $(p_1q_2 + p_2q_1)$ be represented by D, and $(p_1p_2) + (1-s)\ D$ by C, the above fraction becomes $C^2/[C + D(1-s)]^2$.

Summing up the genotypes among the first generation following selection and making the above substitutions by C and D to simplify numerical calculations, the

frequency of gene a becomes $(1-s)(p_2q_1)/C+D(1-s)$, and the frequency of gene b is $(1-s)(p_1q_2)/C+D(1-s)$.

The population with which we started will be designated by the 0 generation. The formulas for all the generations can now be derived by the method described. Let n represent the number of the generations.

FREQUENCY OF GENE a

First generation,

$$\frac{(1-s)(p_2q_1)}{C+D(1-s)}.$$

Second generation,

$$\frac{(1-s)^2(p_2q_1)}{C+2D(1-s)^2}.$$

Subsequent generations,

$$\frac{(1-s)^n(p_2q_1)}{C+D(1-s)^2\ldots D(1-s)^{n-1}+2D(1-s)^n}.$$

FREQUENCY OF GENE b

First generation,

$$\frac{(1-s)(p_1q_2)}{C+D(1-s)}.$$

Second generation,

$$\frac{(1-s)^2(p_1q_2)}{C+2D(1-s)^2}.$$

Subsequent generations,

$$\frac{(1-s)^n(p_1q_2)}{C+D(1-s)^2\ldots D(1-s)^{n-1}+2D(1-s)^n}.$$

FREQUENCY OF GENOTYPE AABB

First generation,

$$\frac{C^2}{[C+D(1-s)]^2}.$$

Second generation,

$$\frac{[C+D(1-s)^2]^2}{[C+2D(1-s)^2]^2}.$$

Subsequent generations,

$$\frac{[C+D(1-s)^2\ldots D(1-s)^n]^2}{[C+D(1-s)^2\ldots D(1-s)^{n-1}+2D(1-s)^n]^2}.$$

Tables 1–8 give the calculated frequencies of genes a and b and of the homozygote AABB for different values of p_1, q_1, p_2, q_2, and s. Selection in the initial (0) generation in all cases is very effective because genotypes AAbb, aaBB, AaBb, Aabb, aaBb, and aabb are discarded entirely.

Tables 1–4 represent populations in which the incidences of gene a ($q_1 = 0.8$) and gene b ($q_2 = 0.9$) are high. These four tables show the consequences of decreasing

rates of selection from $s = 0.7$ to $s = 0.05$. In all cases, genes a and b decrease toward elimination, and the frequency of the homozygote, AABB, approaches 100 per cent as a limit. In other words, highly accurate selection against AABb and AaBB, which enables one to discard 70 per cent ($s = 0.7$) of such individuals reduces both a and b to a frequency of less than 1 per cent in five generations and raises AABB to 99 per cent. In Table 4, only 5 per cent of these genotypes are detected and discarded ($s = 0.05$); nevertheless in something over thirty generations homozygosity is approximated also.

Continuous, though somewhat weak, selection accomplishes in the end the same results as precise selection, but it takes longer. Therefore, increased precision is very desirable, if it is practical.

Tables 5 and 6 deal with populations having intermediate frequencies of a and b, ($q_1 = q_2 = 0.5$). Rigorous selection ($s = 0.8$) attains near-homozygosity in four generations; less intense selection ($s = 0.2$) accomplishes the same result, but it requires more than three times as many generations.

TABLE 1

s=0.7

Generation			*f* of AABB
0.....	p_1=0.2 q_1=0.8	p_2=0.1 q_2=0.9	0.0004
	f Gene a	*f* Gene b	
1.....	0.1364	0.3068	0.3100
2.....	0.0497	0.1119	0.7029
3.....	0.0159	0.0359	0.8990
4.....	0.0049	0.0110	0.9685
5.....	0.0015	0.0033	0.9904

TABLE 2

s=0.5

Generation			*f* of AABB
0.....	p_1=0.2 q_1=0.8	p_2=0.1 q_2=0.9	0.0004
	f Gene a	*f* Gene b	
1.....	0.1429	0.3214	0.2870
2.....	0.0714	0.1607	0.5896
3.....	0.0357	0.0804	0.7813
4.....	0.0179	0.0402	0.8873
5.....	0.0089	0.0201	0.9428
6.....	0.0045	0.0100	0.9712
7.....	0.0022	0.0050	0.9855
8.....	0.0011	0.0025	0.9928

TABLE 3

$s=0.1$

Generation			f of AABB
0.....	$p_1=0.2$ $q_1=0.8$	$p_2=0.1$ $q_2=0.9$	0.0004
	f Gene a	f Gene b	
1.....	0.1475	0.3320	0.2709
2.....	0.0960	0.2159	0.4735
3.....	0.0691	0.1555	0.6012
4.....	0.0527	0.1187	0.6866
5.....	0.0417	0.0939	0.7470
6.....	0.0339	0.0762	0.7919
7.....	0.0280	0.0628	0.8261
8.....	0.0235	0.0529	0.8530
9.....	0.0199	0.0449	0.8746
10.....	0.0171	0.0384	0.8922
11.....	0.0147	0.0331	0.9067
12.....	0.0127	0.0286	0.9189
13.....	0.0110	0.0250	0.9291
14.....	0.0097	0.0218	0.9379
15.....	.....	0.0192	0.9453
16.....	.....	0.0169	0.9518
17.....	.....	0.0149	0.9574
18.....	.....	0.0132	0.9622
19.....	.....	0.0117	0.9665
20.....	.....	0.0104	0.9702
21.....	0.0041	0.0092	0.9735

TABLE 4

$s=0.05$

Generation			f of AABB	Generation	f Gene a	f Gene b	f of AABB
0.....	$p_1=0.2$ $q_1=0.8$	$p_2=0.1$ $q_2=0.9$	0.0004	15.....	0.0133	0.0299	0.9156
				16.....	0.0121	0.0273	0.9226
				17.....	0.0111	0.0251	0.9289
				18.....	0.0103	0.0231	0.9345
				19.....	0.0095	0.0213	0.9395
				20.....	0.0087	0.0197	0.9440
	f Gene a	f Gene b		21.....	0.0081	0.0182	0.9481
1.....	0.1479	0.3327	0.2698	22.....	0.0075	0.0169	0.9518
2.....	0.0988	0.2223	0.4642	23.....	0.0070	0.0157	0.9551
3.....	0.0724	0.1629	0.5848	24.....	0.0065	0.0146	0.9582
4.....	0.0568	0.1277	0.6650	25.....	0.0061	0.0136	0.9610
5.....	0.0462	0.1040	0.7220	26.....	0.0057	0.0127	0.9636
6.....	0.0387	0.0871	0.7643	27.....	0.0053	0.0119	0.9659
7.....	0.0330	0.0743	0.7969	28.....	0.0050	0.0111	0.9681
8.....	0.0286	0.0644	0.8227	29.....	0.0046	0.0104	0.9701
9.....	0.0251	0.0564	0.8436	30.....	0.0043	0.0098	0.9720
10.....	0.0222	0.0499	0.8609				
11.....	0.0198	0.0446	0.8754				
12.....	0.0178	0.0400	0.8878				
13.....	0.0161	0.0361	0.8984				
14.....	0.0146	0.0328	0.9075				

TABLE 5

$s=0.2$

Generation			f of AABB
0.....	$p_1=0.5$ $q_1=0.5$	$p_2=0.5$ $q_2=0.5$	0.0625
	f Gene a	f Gene b	
1.....	0.1905	0.1905	0.3832
2.....	0.1240	0.1240	0.5654
3.....	0.0864	0.0864	0.6844
4.....	0.0626	0.0626	0.7653
5.....	0.0466	0.0466	0.8223
6.....	0.0356	0.0356	0.8638
7.....	0.0271	0.0271	0.8946
8.....	0.0210	0.0210	0.9178
9.....	0.0164	0.0164	0.9356
10.....	0.0129	0.0129	0.9493
11.....	0.0101	0.0101	0.9599
12.....	0.0080	0.0080	0.9682

TABLE 6

$s=0.8$

Generation			f of AABB
0.....	$p_1=0.5$ $q_1=0.5$	$p_2=0.5$ $q_2=0.5$	0.0625
	f Gene a	f Gene b	
1.....	0.1111	0.1111	0.6049
2.....	0.0256	0.0256	0.9000
3.....	0.0053	0.0053	0.9789
4.....	0.0011	0.0011	0.9958

TABLE 7

$s=0.5$

Generation			f of AABB
0.....	$p_1=0.2$ $q_1=0.8$	$p_2=0.9$ $q_2=0.1$	0.0324
	f Gene a	f Gene b	
1.....	0.3913	0.0109	0.3574
2.....	0.1957	0.0054	0.6382
3.....	0.0978	0.0027	0.8091
4.....	0.0489	0.0014	0.9019
5.....	0.0245	0.0007	0.9504
6.....	0.0122	0.0003	0.9751
7.....	0.0061	0.0002	0.9875

Table 7 illustrates what happens when the frequency of a is high, ($q_1 = 0.8$), that of b low, ($q_2 = 0.1$), and the selection pressure fairly intense, ($s = 0.5$).

Table 8 presents a case in which there are three rates of selection instead of two. All animals of the genotypes Aabb, aaBb, and aabb are discarded. Only 60 per cent ($s_2 = 0.6$) of the genotypes AAbb, AaBb, and aaBB are detected and eliminated; 40 per cent are bred. Twenty per cent ($s_1 = 0.2$) of AABb and AaBB are discarded; 80 per cent are bred. Such a situation might arise if the identification of genotypes

TABLE 8

s_1=0.2=SELECTION PRESSURE AGAINST AABb AND AaBB
s_2=0.6=SELECTION PRESSURE AGAINST AAbb, AaBb, AND aaBB
(All Animals of Genotypes Aabb, aaBb, and aabb Are Discarded.)

Generation			*f* of AABB
0.....	p_1=0.6 q_1=0.4	p_2=0.7 q_2=0.3	0.1764
	f Gene a	*f* Gene b	
1.....	0.2720	0.1867	0.3311
2.....	0.2021	0.1333	0.4623
3.....	0.1536	0.0990	0.5712
4.....	0.1179	0.0748	0.6593
5.....	0.0914	0.0570	0.7293
6.....	0.0714	0.0443	0.7852
7.....	0.0559	0.0342	0.8291
8.....	0.0439	0.0267	0.8642
9.....	0.0346	0.0210	0.8919
10.....	0.0274	0.0168	0.9143
11.....	0.0219	0.0134	0.9319
12.....	0.0174	0.0106	0.9436
13.....	0.0139	0.0083	0.9561
14.....	0.0110	0.0066	0.9648

was seriously hampered by modifying genes, lack of consistency in preparing rations, considerable variation in the oral flora, etc. Yet homozygosity is theoretically approached in spite of these difficulties.

A general formula was not derived to apply to cases with selection rates s_1 and s_2, but the data in Table 8 were obtained by arithmetic, which is a laborious process.

To summarize: Building a uniform (near-homozygous) stock of caries-resistant animals is difficult. Progeny testing, establishment of the oral flora, adequate attention to diet, and improving the technique for identifying cavities in the teeth should be parts of the procedure. Selective breeding, if persistently pursued, will produce genetically uniform stock, even if selection lacks high intensity.

Caries Patterns of Intact and Sialoadenectomized Albino Rats of the Emory Wistar Strain Fed a Cariogenic Diet

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The susceptibility of the albino rat to dental caries is highly variable, being dependent on genetic background, composition of the diet, housing, method of feeding, and other factors. Our colony, the Emory Wistar strain, has been inbred in our laboratories for the last 25 years. Our stock animals are maintained on Purina laboratory chow and are relatively caries-resistant. A number of investigators have developed strains of albino rats that exhibit various degrees of susceptibility or resistance to dental caries; among these are Shaw at the Harvard School of Dental Medicine; Hunt and his co-workers at Michigan State University; and Losee at the National Medical Research Institute.

Most investigators report that in the albino rat there is no sex difference in susceptibility to dental caries;[1–4] however, there are some who find a sex difference in caries incidence in their animals.[5, 6]

A high degree of bilateral symmetry in dental caries experience in man and animals has been reported in the literature.[1, 2, 5, 7, 8] However, Wheatcraft and his associates[5] draw attention to the fact that the bilateral symmetry is not perfect and that, although it is nearly perfect when dealing with average values, there are differences in individual animals.

In studies conducted in our laboratories on the relative cariogenicity of high-sucrose diets and on the role of minerals in the caries conduciveness of synthetic diets, we have used a large number of sialoadenectomized albino rats. The reason for the removal of the major salivary glands was to reduce the length of time required for the development of moderate to severe caries in our relatively resistant strain of animals. The present experiment was conducted to compare the caries patterns and the incidence, location, and extent of the caries in intact and sialoadenectomized animals of our colony. Since the Emory Wistar strain of rats does not develop smooth-surface caries when fed the cariogenic diet used in this experiment, this study was made on occlusal-fissure caries only.

EXPERIMENTAL METHODS

Twenty littermate pairs of Emory Wistar albino rats were selected at weaning, ten pairs of males and ten pairs of females. One of each pair was subjected to a sham

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operation, and the other was sialoadenectomized by our usual procedure.[9] The animals were fed the Harvard 100 diet, which is a high-sucrose synthetic diet containing 64 per cent sucrose, 23 per cent casein, 5 per cent corn oil, 4 per cent liver concentrate, and 4 per cent salt mixture with added vitamins. The detailed composition of the mineral and vitamin content may be found elsewhere.[10] The animals were allowed to eat and drink ad libitum. At the end of a 70-day experimental period, the animals were sacrificed, and the mandibles and maxillae were cleaned and dried. The teeth were scored for incidence and extent of carious lesions by our usual procedure. The teeth were examined under a dissecting microscope, and the scores were recorded as follows: grade 1—initiation of caries, as indicated by the characteristic brown stain without any definite cavitation; grade 2—cavity large enough to admit a small explorer; grade 3—moderate spread of the lesion, involving the cusps; grade 4—spread of the lesion from one fissure to another by the breakdown of the cuspal walls; grade 5—spread of the lesion to the extent that the involved area (which might be the entire tooth) was lost.

TABLE 1

OCCLUSAL FISSURE CARIES IN MOLARS OF SHAM-OPERATED AND SIALOADENECTOMIZED ALBINO RATS FED HIGH-SUCROSE DIET

QUADRANT	SHAM-OPERATED*		SIALOADENECTOMIZED*	
	Av. No. Lesions±S.E.M.	Av. Caries Scores±S.E.M.	Av. No. Lesions±S.E.M.	Av. Caries Scores±S.E.M.
Right mandible	2.7±0.4	3.5±0.5	6.5±0.2	14.1±0.9
Left mandible	2.7±0.3	3.4±0.5	6.6±0.2	14.1±1.1
Right maxilla	1.2±0.3	1.2±0.3	5.2±0.2	9.0±0.9
Left maxilla	1.4±0.3	1.5±0.4	5.2±0.2	8.9±1.0
Right and left mandibles	5.4±0.6	6.9±0.9	13.1±0.3	28.2±2.3
Right and left maxillae	2.6±0.7	2.7±0.7	10.4±0.7	17.9±1.5
All molars	8.0±1.2	9.6±1.6	23.5±0.5	46.1±3.5

* 20 animals in each group.

Since there is a total of six fissures in the three teeth of each quadrant, the maximum number of lesions that can be obtained is 6×4, or 24. The caries scores are obtained by multiplying the number of lesions found by the grade (1 through 5) of the lesions. For example, if an animal was found to have 6 lesions grade 1, the score would be $6 \times 1 = 6$, or if there were 6 lesions, 3 of which were grade 1 and 3 were grade 2, the score would be $3 \times 1 + 3 \times 2 = 9$.

RESULTS

Examination of the teeth of the animals in this experiment failed to reveal evidence of any smooth-surface caries in either the sham-operated or the sialoadenectomized groups. The Emory Wistar strain does not develop smooth-surface caries when fed the Harvard 100 diet in our laboratories. Therefore, the data presented here are on the incidence and extent of occlusal-fissure caries only.

The data presented in Table 1 give the average number of carious lesions and the average caries scores of the sham-operated and sialoadenectomized animals. The scores on the teeth of the males and females were averaged together, as there was no

difference in the averages of these two groups. The data are given for the incidence and extent in each mandible and maxilla, for the right and left mandibles together and the right and left maxillae together, and also for the sum of all the molars. The average number of lesions in all the molars of the sham-operated animals is relatively small, only 8 out of 24, or one-third of the fissures, presenting evidence of caries. The average caries score of 9.6 indicates that most of the lesions were grade 1, with only a few graded 2, so that most of the caries found in these animals was of the incipient kind, in that no cavitation had occurred.

On the other hand, in the sialoadenectomized littermates of the sham-operated animals, there was severe decay. In Table 1 it may be seen that the average number of lesions in all the molars for each rat was 23.5, as compared with 8 for the controls. This value indicates that practically all the fissures of each rat in this group exhibited some degree of caries, as the total number of fissures in the teeth of the albino rat is only 24. It is also shown in Table 1 that the average extent of each lesion was greater in the sialoadenectomized than in the sham-operated controls. The average grade or extent of the lesions amounted to approximately 2, while in the sham-operated littermates the average grade or extent was approximately 1. In view of the larger number of lesions and the greater extent of the lesions in the sialoadenectomized animals, the total amount of decay in the teeth of these animals was more than four times that in their sham-operated littermates, the average caries scores for all the teeth being 46.1 and 9.6, respectively.

The number of lesions and the extent of the lesions were much greater in the mandibular teeth than in the maxillary teeth. Also, in agreement with data presented in the literature,[1, 2, 5] the values given in Table 1 exhibit an almost perfect bilateral symmetry in the caries patterns of both the sham-operated and the sialoadenectomized animals.

A statistical analysis of the data in Table 1 on the average number of carious lesions and the average caries scores for the sum of the right and left mandibular molars and for the sum of the right and left maxillary molars, as well as for the sum of all the molars, is presented in Table 2. This analysis shows that the differences obtained in the incidence and extent of caries in the intact and sialoadenectomized animals were highly significant, with critical ratios of 12.0 and 9.4, respectively. Also, the differences in susceptibility between the mandibular and the maxillary molars are statistically significant, the critical ratios ranging from 3.0 to 6.3. It was not deemed of sufficient interest to report a statistical study on the individual mandibles and maxillae.

In Tables 3 and 4 will be found a comparison of the average caries scores for each fissure and each tooth in the intact and sialoadenectomized animals. These data further indicate a very high degree of bilateral symmetry in the caries patterns of both groups of rats.

The most severe lesions occurred in the second fissure of the first mandibular molar; the second most severe, in the first fissure of the second mandibular molar. It may also be seen from Table 3 that very little decay occurred in the third molars.

DISCUSSION

A number of investigators have reported that caries-resistant albino rats and hamsters become caries-susceptible upon removal of the salivary glands. Also, caries-

susceptible animals with salivary glands intact exhibit less decay than their littermates which have had their salivary glands removed. It seems, however, that the effect on caries brought about by removal of the major salivary glands varies from one laboratory to another. For example, the incidence and extent of caries which were brought about by removal of the major salivary glands in the present study were much greater than those reported by Bixler, Muhler, and Shafer.[3] These investigators reported an increase in caries from 55 to 80 per cent in sialoadenectomized rats, as compared with the 300–400 per cent increase found in our animals. The Indiana strain of

TABLE 2

STATISTICAL ANALYSIS OF CARIES INCIDENCE AND CARIES SCORES

	Sham-operated		Sialoadenectomized	
	Av. No. Lesions	Av. Caries Scores	Av. No. Lesions	Av. Caries Scores
Mandibular teeth	5.4±0.6*	6.9± 0.9	13.1±0.3	28.2±2.3
	3.0†	3.7	6.3	3.6
Maxillary teeth	2.6±0.7	2.7± 0.7	10.4±0.3	17.9±1.5
Mandibular and maxillary teeth	8.0±1.2	9.6± 1.6	23.5±0.5	46.1±3.5
		12.0	9.4	

* Standard error of the mean.

† Critical ratio. The critical ratio (C.R.) is the ratio of the difference between two means to the standard error of the difference between the means. When the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; 2.0 to 2.9, the difference is of borderline significance; 3.0 or more, the difference is highly significant. (J. M. Dunning, *J. D. Res.*, **29**:541, 1950).

TABLE 3

AVERAGE CARIES SCORES FOR EACH FISSURE IN MOLAR TEETH OF SHAM-OPERATED AND SIALOADENECTOMIZED RATS FED HIGH-SUCROSE DIET

	Fissure*											
	A	B	C	D	E	F	A	B	C	D	E	F
	Right Mandibles						Left Mandibles					
Sham-operated	0.3	1.4	0.7	1.1	0.1	0.05	0.3	1.2	0.8	1.0	0.2	0.1
Sialoadenectomized	2.5	3.0	2.1	2.6	1.7	1.0	2.5	3.0	2.3	2.5	1.5	1.0
	Right Maxillae						Left Maxillae					
Sham-operated	0.2	0.4	0.2	0.4	0.05	0.0	0.3	0.3	0.4	0.5	0.05	0.0
Sialoadenectomized	1.9	2.0	1.9	2.1	0.9	0.4	1.9	2.1	1.7	1.8	0.8	0.4

* *Mandibular teeth*—fissures A, B, and C comprise first molar; D and E, second molar; and F, third molar. *Maxillary teeth*—fissures A and B comprise first molar; C and D, second molar; and E and F, third molar.

rats is evidently not very susceptible to caries, even though the animals were sialoadenectomized; also, the method of removal of the major salivary glands may be different from ours.

TABLE 4

AVERAGE CARIES SCORES FOR EACH MOLAR TOOTH OF SHAM-OPERATED AND SIALOADENECTOMIZED RATS FED HIGH-SUCROSE DIET

	Molar No.					
	First	Second	Third	First	Second	Third
	Right Mandibles			Left Mandibles		
Sham-operated	2.4	1.2	0.05	2.3	1.0	0.1
Sialoadenectomized	7.6	4.3	1.0	7.8	4.0	1.0
	Right Maxillae			Left Maxillae		
Sham-operated	0.6	0.6	0.05	0.6	0.9	0.05
Sialoadenectomized	3.9	4.0	1.3	4.0	3.5	1.2

SUMMARY

In agreement with the report of Muhler and Bixler,[11] we found that sialoadenectomized rats fed a high-sucrose diet developed much more caries in the mandibular teeth than in the maxillary teeth.

The locations of the most severe and least severe lesions as to fissure and tooth found in our study are in agreement with the report of Dalderup.[1] It may be of interest that we, as well as Dalderup, rarely find lesions in the third molars. This very low degree of susceptibility of the third molars differs from that seen by Volker[12] in hamsters. Volker found the most severe decay in the third molars.

Our procedure has been to start feeding our animals the cariogenic diets at 21–25 days of age, and, since the Emory Wistar strain, when sialoadenectomized, develops severe caries in the first and second molars in 70 days, we ordinarily terminate the experiment at this time. One possible explanation of the relatively low incidence and extent of the caries found in the third molars of our strain may be due to the fact that the third molars erupted 10–14 days after the experiments had been started and were therefore exposed to the cariogenic diet a shorter time than the first and second molars. Perhaps this shorter exposure time is not sufficiently long to permit any appreciable development of lesions in these teeth.

In our several experiments feeding the Harvard 100 diet, we have never observed any sex difference in our animals in the susceptibility to caries. To check this observation, 100 males and 100 females that had been sialoadenectomized and fed this diet for 70 days were selected in equal numbers from various experiments. The average caries scores of the animals were 46 for the males and 44 for the females. It is of interest that

in 9 experiments conducted over a period of 10 years, various groups of animals ranging from 20 to 25 in number exhibited average caries scores of from 39 to 46, with an over-all average of 43. These data indicate that the Emory Wistar strain of albino rats gives reproducible results in studies on dental caries.

SUMMARY

Twenty littermate pairs of Emory Wistar strain albino rats were selected at weaning, ten pairs of males and ten pairs of females. One of each pair was subjected to a sham operation, and the other was sialoadenectomized. All animals were fed a highly cariogenic high-sucrose diet for a 70-day period, at the end of which time the animals were sacrificed and the teeth scored for caries.

Removal of the major salivary glands of the rats of the Emory Wistar strain resulted in an increase in incidence and extent in caries of between 300 and 400 per cent. There was a high degree of bilateral symmetry in incidence and extent of caries in both groups. Observations of the caries in a large group of animals in this strain showed no sex difference in susceptibility to caries. The Emory Wistar strain of albino rats gives reproducible results in caries studies.

REFERENCES

1. Dalderup, L. M. *Nutrition and Caries,* p. 24. Amsterdam: Drukkerij Verdonk, 1959.
2. Shaw, J. H., and Sweeney, E. A. Observations on Sexual Similarity and Bilateral Distribution in Dental Caries Incidence in Albino Rats and Cotton Rats, *J. D. Res.*, **35**:286, 1956.
3. Bixler, D., Muhler, J. C., and Shafer, W. G. The Effects of Castration, Sex Hormones and Desalivation on Dental Caries in the Rat, *J. D. Res.*, **34**:889, 1955.
4. Sognnaes, R. F. Experimental Rat Caries. II. Location, Sequence and Extent of Carious Lesions Produced in the Norway Rat when Raised on a Generally Adequate, Finely Powdered Purified Ration, *J. Nutrition,* **39**:139, 1949.
5. Wheatcraft, M. G., Gerende, L. J., Schlack, C. A., Taylor, B. L., Berzinskas, V. J., and Mullins, C. E. Bilateral Symmetry of Dental Caries, *J. D. Res.*, **30**:62, 1951.
6. Stelling, E., and Gustafson, G. Experimental Dental Caries and Hormones, *Odont. Revy,* **7**:354, 1956.
7. McCauley, H. B., and Dale, P. P. Observations of Increased Caries Activity Following Interruption of Topical Fluorine Applications, *J. D. Res.*, **24**:305, 1945.
8. Bibby, B. G., and Tursky, S. S. A Note on the Duration of Caries Inhibition Produced by Fluoride Applications, *J. D. Res.*, **26**:105, 1947.
9. Haldi, J., Wynn, W., Shaw, J. H., and Sognnaes, R. F. The Relative Cariogenicity of Sucrose when Ingested in the Solid Form and in Solution by the Albino Rat, *J. Nutrition,* **49**:295, 1953.
10. Wynn, W., Haldi, J., Shaw, J. H., and Sognnaes, R. F. Differences in the Caries-producing Effects of Two Purified Diets Containing the Same Amount of Sugar, *J. Nutrition,* **50**:267, 1953.
11. Muhler, J. C., and Bixler, D. Decreased Activity of Dental Caries in Desalivated Albino Rats, *J. D. Res.*, **35**:615, 1956.
12. Volker, J. F. Some Observations concerning Dental Caries Production in the Syrian Hamster, *J. D. Res.*, **30**:484, 1951.

Studies on the Intermediary Carbohydrate Metabolism in Rat Oral Mucosa

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During recent years there has been an increasing appreciation of the metabolic mechanisms that occur in the oral mucosa. Recent advances in histochemical and cytochemical techniques and the fact that inflamed oral tissues have an increased rate of oxygen consumption in vitro,[1–4] have stimulated investigation of the intermediary metabolism of the oral mucosa.

The results of many histochemical[5–10] and cytochemical studies[11–16] have suggested that the Embden-Meyerhof pathway (EMP) may be functional in the oral soft tissues of various animals. Other investigations suggest that the tricarboxylic acid cycle,[11–14, 17–19] the hexose monophosphate pathway,[20, 21] and pathways for fatty acid and amino acid oxidation[12] may be operative in animal oral mucosa.

This report concerns the in vitro investigations of the intermediary metabolic pathways in rat oral mucosa as indicated by alterations in the oxygen uptake of the tissue produced by the addition of various intermediary metabolic materials of the Embden-Meyerhof pathway and the tricarboxylic acid cycle.

MATERIALS AND METHODS

The oxygen uptake was measured by the interval uptake modification of the direct method of Warburg as outlined by Umbreit, Burris, and Stauffer.[22] The tissue used was the soft tissue covering the hard and soft palates of 4- to 5-month-old white rats of the St. Louis University strain. Histologic examination indicated that the specimen consisted of keratinized, stratified, squamous epithelium, collagenous connective tissue, and a small amount of mucous glandular tissue.

The suspending medium was Krebs-Ringer-phosphate, pH 7.4, with antibiotics added to inhibit bacterial proliferation; the final concentration of the antibiotics was 50 I.U. of procaine penicillin/ml and 50 μg of dihydrostreptomycin/ml. The suspending solution was gassed with oxygen for 10 minutes shortly before use.

Commercially obtainable intermediary materials of the Embden-Meyerhof pathway and the tricarboxylic acid cycle were added to the suspending solution as one-sixth of the final volume, so that the final concentration in the main compartment of the Warburg flask was 0.04 M. The Embden-Meyerhof pathway intermediary materials used

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were salts of glucose-1-phosphate, glucose-6-phosphate, fructose-1,6-diphosphate, and pyruvic acid. In addition, glucose, galactose, fructose, mannose, and lactate were also tested. Adenosine triphosphate was added to one trial with glucose. The intermediary materials of the tricarboxylic acid cycle used were salts of citric acid, isocitric acid, ketoglutaric acid, succinic acid, fumaric acid, malic acid, and oxaloacetic acid. In addition, salts of acetic acid and glutamic acid were also tested.

The tissue specimens were approximately 1 mm. in thickness and well within the maximum thickness allowable by Warburg's formula.[22] The manometers and flasks were flushed with oxygen for 10 minutes and allowed to equilibrate at 37° ± 0.01° C. for 30 minutes. Readings were taken at hourly intervals, and the Q_{O_2} (μl oxygen uptake/mg wet weight) was calculated for each specimen.[2] Using the "*t*"-test, statistical comparisons were made between specimens with added substrates and endogenous controls. Only the results of the intermediary materials that stimulated the Q_{O_2} are shown.

RESULTS

Of the Embden-Meyerhof pathway and closely related intermediary materials, only fructose and fructose-1,6-diphosphate stimulated the uptake of oxygen (Figs. 1 and 2, respectively). To test the possibility that an exogenous source of adenosine triphosphate was required for the metabolism of glucose, 1.3×10^{-3} M adenosine triphosphate[24] was added to the suspending solution. The addition of adenosine triphosphate decreased the oxygen uptake of the tissue (Fig. 3).

Since it is known that pyruvic acid is metabolized slowly, it has been recommended that an exogenous source of oxaloacetic acid be added to stimulate pyruvic acid oxidation.[22] A 0.04 M solution of fumaric acid was added as the source of oxaloacetic acid, and this combination of intermediary materials stimulated the Q_{O_2} (Fig. 4). Since the stimulation may have been due to the fumaric acid alone, the results were compared with the combination of pyruvic and fumaric acid, using fumaric acid as a parameter (Fig. 5). The comparison indicated that pyruvic acid increases the oxygen uptake of the rat palate.

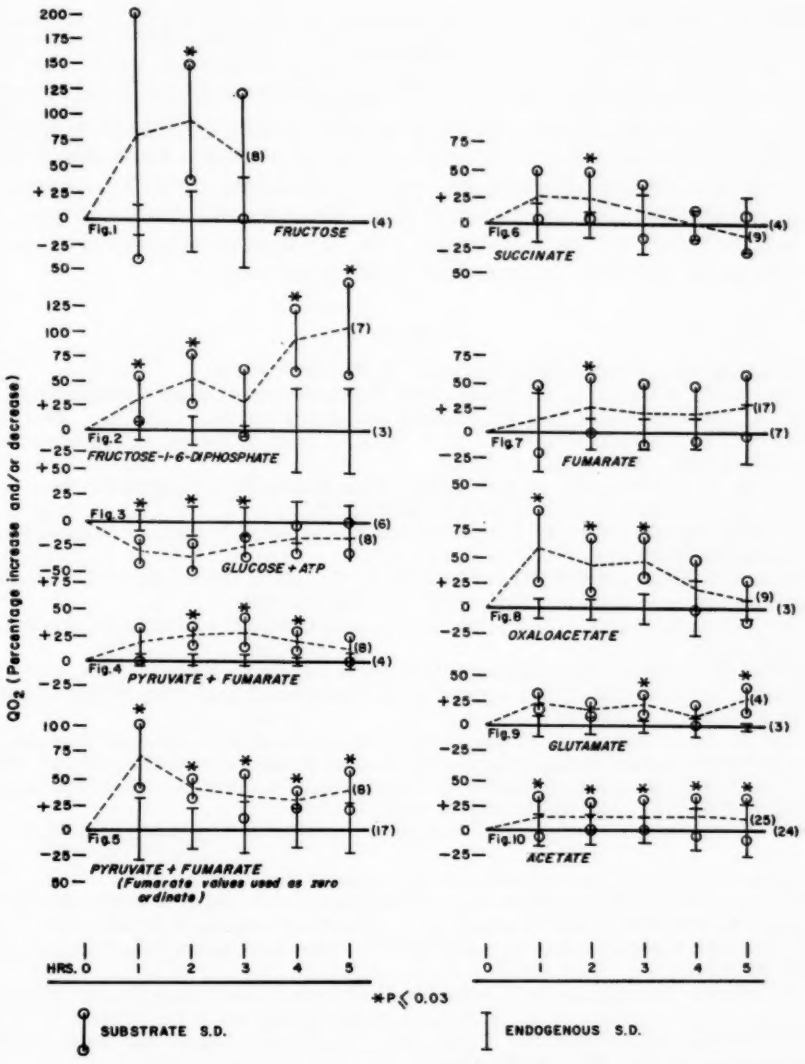
Of the tricarboxylic acid cycle intermediary compounds tested, only succinic, fumaric, and oxaloacetic acid stimulated the oxygen uptake (Figs. 6–8). It is known that the oxidation of malic acid may be inhibited by the accumulation of oxaloacetic acid.[22] The glutamic acid–malic acid combination did not increase the oxygen uptake over glutamic acid controls; however, glutamic acid alone did increase the Q_{O_2} (Fig. 9). Acetic acid also increased the Q_{O_2} of the rat palate (Fig. 10).

DISCUSSION

A significant increase in oxygen uptake of a tissue specimen suggests that the enzyme required for the metabolism of a particular intermediary material is functional within the tissue.[25] The increase in oxygen uptake produced by fructose, fructose-1,6-diphosphate and pyruvic acid suggests that the Embden-Meyerhof pathway may be functioning in rat oral mucosa. These results are similar to those obtained by other investigators.[11-14] The decrease in oxygen uptake produced by adenosine triphosphate is not well understood but may be a manifestation of the Crabtree effect.[26]

If this is the case, it suggests that a glycolytic pathway, presumably the Embden-Meyerhof pathway, is functional in rat oral mucosa.

The results obtained with succinic acid, fumaric acid, oxaloacetic acid, acetic acid, and glutamic acid suggest that several enzymes of the tricarboxylic acid cycle are functional in rat oral mucosa. With the additional evidence that isocitric dehydrogenase



FIGS. 1–10.—In the above figures (with the exception of Fig. 5) the zero ordinate represents the endogenous control values. The broken line shows the percentage increase or decrease in the QO_2 produced by the indicated substrate. The numbers in parentheses at the end of the ordinates indicate the specimens used in each particular trial. The "*t*"-test was used to calculate statistical significance.

has been detected in rat palate,[11] it appears quite likely that the tricarboxylic acid cycle is operative in this tissue.

The inability of the other intermediary substrate materials to stimulate the oxygen uptake is not well understood. Several possible explanations are the inability to diffuse into the cell, possibly due to the absence of specific cell membrane "permeases";[27] the accumulation of inhibiting substance within the cell;[22] and the inhibition of cellular energy.[26]

SUMMARY

Various intermediary materials of the Embden-Meyerhof pathway and the tricarboxylic acid cycle, plus closely related intermediary materials, were investigated for the ability to stimulate the in vitro oxygen uptake of rat palatal tissue. The following substrates were effective: fructose, fructose-1,6-diphosphate, pyruvate, succinate, fumarate, oxaloacetate, acetate, and glutamate. These results, along with previously reported findings in the literature, suggest that the Embden-Meyerhof pathway and the tricarboxylic acid cycle are functional in rat oral mucosa.

REFERENCES

1. Cattoni, M. Oxygen Uptake of the Gingiva, *J. D. Res.*, **33**:695, 1953.
2. Glickman, I., Turesky, S., and Hill, R. The Determination of Oxygen Consumption in Normal and Inflamed Gingiva Using the Warburg Manometric Technique, *J. D. Res.*, **28**:83, 1949.
3. Schrader, H., Schrader, R., and Ott, G. A. Oxygen Uptake by Normal and Inflamed Gingiva and Saliva, *Helv. Odont. Acta*, **1**:650, 1957.
4. Schrader, H., and Schrader, R. Examination of the Metabolism of Gingiva and Saliva in Patients Afflicted with Periodontosis, *D. Abs.*, **2**:650, 1957.
5. Dewar, M. Observations on the Composition and Metabolism of Normal and Inflamed Gingiva, *J. Periodont.*, **26**:29, 1955.
6. Forscher, B. K., Paulsen, A. G., and Hess, W. C. The pH of the Periodontal Pocket and the Glycogen Content of Adjacent Tissue, *J. D. Res.*, **33**:444, 1954.
7. ———. Glycogen Content of Periodontal Tissues. II. Correlation with Tyrosine Content, *ibid.*, **34**:144, 1955.
8. Trott, J. R. Presence and Distribution of Glycogen in the Gingiva, *Austral. J. Den.*, **2**:283, 1957.
9. Turesky, S., Glickman, I., and Litwin, T. A Histochemical Evaluation of Normal and Inflamed Gingiva, *J. D. Res.*, **30**:792, 1951.
10. Weiss, M. D., Weinmann, J. P., and Meyer, J. Degree of Keratinization and Glycogen Content in Uninflamed and Inflamed Gingiva and Alveolar Mucosa, *J. Periodont.*, **30**:208, 1959.
11. Masui, Y. Dehydrogenase of Gingiva. I. Dehydrogenase Activities in Normal Rat and Dog Gingiva, *Kokubyo-Gakkai Zasshi*, **25**:554, 1958.
12. ———. Dehydrogenase of Gingiva. II. Dehydrogenase Activities with Substrate Addition, *ibid.*, p. 563.
13. Kinoshita, S. Respiration of Gingiva, *Kokubyo-Gakkai Zasshi*, **26**:99, 1959.
14. Zar, I., and Irving, J. T. The Metabolism of Sheep Gingiva, *J. S. African Med. Soc.*, **22**:102, 1958.
15. Eichel, B., Pena, H. E., and Swanson, A. A. Intracellular Oxidative Enzymes in Oral Tissues. III. Spectrophotometric Determination of Pyruvic Reductase (Lactic Dehydrogenase), *J. D. Res.*, **34**:682, 1955.
16. ———. Pyruvic Reductase in Oral, Ocular, Liver, and Brain Cortex Tissues, *D. Abs.*, **3**:598, 1958.
17. Eichel, B., and Swanson, A. A. Intracellular Oxidative Enxymes in Oral Tissues. II. Succinic Dehydrogenase, DPN-Cytochrome C Reductase, Cytochrome Oxidase, and Total Protein in Tongue Mucosa, *J. D. Res.*, **34**:682, 1955.
18. Eichel, B., Swanson, A. A., and Bradley, T. Oxidative Enzymes. I. Succinic Dehydrogenase, DPN-Cytochrome C Reductase, Cytochrome Oxidase, and Catalase in Oral, Liver, and Brain Cortex Tissues, *J. D. Res.*, **36**:581, 1957.
19. Person, P., and Burnett, G. W. Dynamic Equilibria of Oral Tissues. II. Cytochrome Oxidase and Succinoxidase Activity of Oral Tissues, *J. Periodont.*, **26**:99, 1955.

20. EICHEL, B. The Labile Nature and Mode of Protection of Glucose-6-Phosphate Dehydrogenase in Oral Tissues, *I.A.D.R.*, **34**:22, 1956.
21. ———. Intracellular Oxidative Enzymes in Oral Tissues. II. Glucose-6-Phosphate Dehydrogenase in Submaxillary Gland, Gingiva, Tooth Pulp, and Tongue Mucosa, *ibid.*, p. 683, 1955.
22. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F. *Manometric Techniques.* Minneapolis: Burgess Publishing Co., 1957.
23. LUNDQUIST, E. F. *Statistical Analysis in Educational Research.* New York: Houghton Mifflin Co., 1940.
24. GRIESMER, R. D., and GOULD, E. A Method for the Study of the Intermediary Metabolism of Epidermis. I. Oxidation of Acids of the Citric Acid Cycle, *J. Invest. Dermatol.*, **22**:299, 1954.
25. KREBS, H. A. The Regulation of Metabolic Processes, *Texas Repts. Biol. and Med.*, **17**:16, 1959.
26. RACKER, E., and GATT, S. Interactions of Glycolysis and Oxidative Pathways, *Ann. N.Y. Acad. Sci.*, **72**:742, 1959.
27. COHEN, G. N., and RICKENBERG, H. V. Concentration spécifique reversible des amino acides chez *E. coli, Ann. Inst. Pasteur,* **91**:693, 1956.

Histological, Histochemical, and Electron-microscopic Observations on the Postnatal Development of the Major Sublingual Gland of the Rat

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Many problems with regard to the salivary glands and their secretions are occupying the attention of both morphologists and physiologists at the present time. One interesting aspect of this research has been the recognition that in some animals salivary glands are very immature at birth and that full development may occupy a considerable postnatal period. Burgen[1] has drawn attention to this finding in a recent review article. In the rat submaxillary gland, Jacoby and Leeson[2] have found that acini are not present at birth and that the intralobular ducts end in a branching system of terminal tubules. From the first postnatal week on, acini are budded off from the terminal tubules, but they do not reach their full development until the rat is 6–8 weeks old. A similar prolonged postnatal period of development of acini occurs in mice (Jacoby)[3] and in dogs (Wechler unpublished, see Burgen[1]). Burgen has pointed out that "it is remarkable that these very young animals are able to secrete saliva at almost the same rate as the adult animals although there are differences in composition." Indeed, in the rat, the four major salivary glands (the two submaxillary and the two major sublingual glands) are necessary for life. In 1938, Plagge[4] made the interesting observation that removal or duct ligation of these glands resulted in the death of newborn rats within 5 days, due to inanition following mechanical feeding difficulties.

Each major sublingual gland in the rat lies closely applied to the anterolateral surface of the submaxillary gland and, at first sight, appears to be a somewhat separated lobe of the main glandular mass. However, the sublingual glands are paler in color than the submaxillary glands and have separate ducts which open independently into the mouth cavity. Little work has been reported on the histology and cytology of these glands, but recently the electron-microscopic appearances of the adult gland have been described by Scott and Pease,[5] who enumerated the typical features of the mucous acinar cell and of the serous demilune cell. They concluded that the secretory granules of mucous cells arose from the Golgi microvescicles but could find no similar relationship in serous cells.

We have been able to find no report in the literature on the postnatal development of this gland, and the present work was undertaken with this in view and with the hope that we would be able to relate histological and histochemical findings to the fine structural details as revealed by the electron microscope.

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MATERIALS AND METHODS

The series included glands removed from rats differing in age by a week and embraced the period from 1 day to 10 weeks. For each stage, at least two glands were examined. The animals were killed by a blow on the head. For light microscopy, the major sublingual gland was removed, together with a portion of the submaxillary gland, the latter serving as a convenient control to check with previous results reported for this gland.[2] The following fixatives were employed: 80 per cent alcohol and alcoholic Bouin solution. Serial sections from the paraffin-imbedded material were cut at 6 μ, and a series from glands of different ages was mounted in chronologic order on the same slide. This was done not only to facilitate histological comparison of the various stages but also, to insure similar staining conditions throughout a series. The following staining techniques were used: hematoxylin and eosin, van Gieson, picro-Mallory, silver impregnation, alcian blue, azure A (before and after sulphation), periodic acid–Schiff (before and after saliva digestion), and bromophenol blue.

TABLE 1

MEASUREMENTS OF ACINAR AND INTRALOBULAR DUCT DIAMETERS*

Age	Acini	Ducts	Age	Acini	Ducts
Newborn.....	28.0	32.3	5 weeks.....	48.0	48.6
1 week.....	29.3	30.2	6 weeks.....	47.6	50.0
2 weeks.....	29.0	30.0	8 weeks.....	48.6	51.0
3 weeks.....	30.3	35.0	10 weeks.....	50.6	51.1
4 weeks.....	43.0	47.2			

* Each figure, in microns, is an average of 100 measurements.

For electron-microscopic examination, the glands were covered with fixative and cut into small cubes less than 1 mm.[3] in size. The fixative used was 1 per cent osmium tetroxide,[6] and the tissues were fixed for 1 hour. They were then washed in distilled water and dehydrated through graded alcohol. Following infiltration with several changes of 20 per cent methyl in *n*-butyl methacrylate, to which 1 per cent benzoyl peroxide had been added as catalyst, they were imbedded in this medium in gelatin capsules, and polymerized at 60° C. for 16 hours. Sections were cut on an ultramicrotome* with diamond knives and examined in a 75B electron microscope.† Micrographs were taken at initial magnification of 2,000–12,000 diameters and enlarged photographically as desired.

RESULTS

Newborn.—In contrast with the submaxillary gland, which is very immature at birth, the sublingual gland in the newborn rat had the general over-all appearance of the adult. Glandular lobules, separated by loose connective tissue, were clearly defined. Acini were well developed, although they were smaller in size than in the adult (see Table 1) and were more loosely arranged within the lobule (Fig. 1). Nuclei were basally situated in the acinar cells, and the apical cytoplasm was filled with secretory material which stained positively with alcian blue, azure A, and PAS. The adult ap-

* Philips (type 39983). † Philips (type 11981).

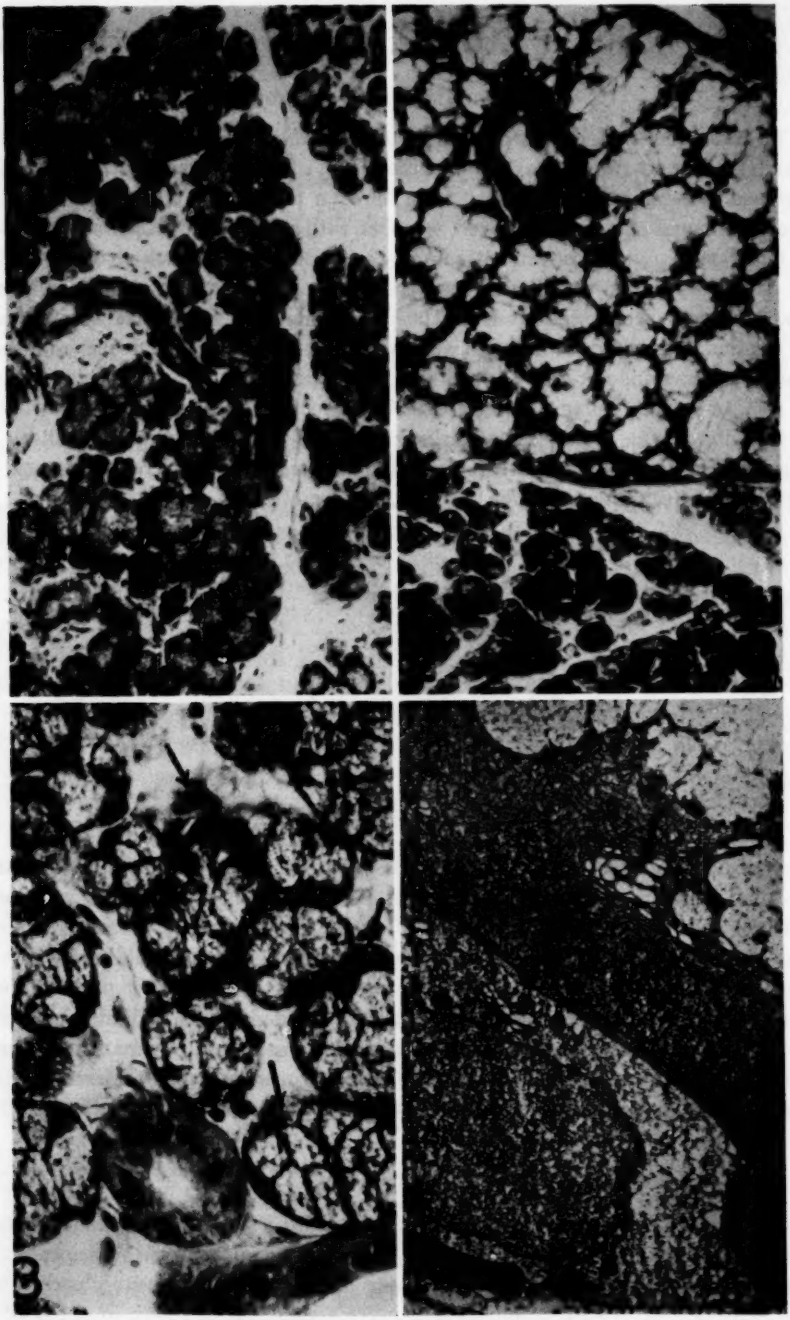

FIG. 1.—Optical micrograph of rat sublingual gland at birth. A portion of the submaxillary gland is present along the right border. (Alcian blue; mag. ×280.)

FIG. 2.—Optical micrograph of 4-week-old sublingual gland to show portions of both the sublingual (*upper*) and the submaxillary (*lower*) glands. (Picro-Mallory; mag. ×500.)

FIG. 3.—Optical micrograph of sublingual gland from 10-week-old rat. Serous cells (*arrowed*) and a cross-section of a duct (*lower center*) are present, in addition to mucous acini. (Alcian blue; mag. ×750.)

FIG. 4.—Electron micrograph of portions of a mucous acinar cell (*above*) and a myoepithelial cell (*below*). The Golgi zone of the acinar cell is arrowed. (Mag. ×13,500.)

pearance of acinar cells was confirmed by electron microscopy. Most of the cell organelles were located basally, and the apical portion of the cell contained the ill-defined secretory material (Fig. 4). The electron dense nucleus lay within the basal cytoplasm, surrounded by closely packed profiles of endoplasmic reticulum. Numerous oval or round mitochondria were also found in this region. In most cells, a prominent Golgi zone was found in a supra-nuclear position. Scott and Pease[5] described this zone in detail in the adult gland and traced the secretory product back directly to Golgi vesicles. Our results were in agreement with this finding.

Also present, as components of some acini, were scattered cells which did not give positive staining reactions for mucus. They were the serous demilune cells. The nuclei of these cells were rounded, and the cytoplasm was strongly acidophilic and contained discrete zymogen granules. Under the electron microscope, the granules of serous cells appeared faintly osmiophilic and homogeneous (Fig. 5). The Golgi apparatus was, in general, less well developed than in mucous cells.

Myoepithelial cells (basket cells) were already present around the acini at birth. These cells could be demonstrated readily by the alkaline phosphatase reaction,[7] which outlined clearly the numerous branching processes embracing the acini. Under the electron microscope, these cells were characterized by a central area containing the nucleus and the majority of cell organelles (Fig. 4) and processes in which the cytoplasm was fibrillar (Fig. 8) and very like that of smooth muscle. These cells lay immediately applied to grandular cells, within the basement membrane of the acinus.

Myoepithelial cells, less complex in their branching processes, were also present around intercalated ducts, which appeared adult in form. Component cells of these ducts were characterized by the presence of interlocking at the interphase between cells and small microvilli along the luminal border (Fig. 10). The basal-cell membrane was without specialization. Intralobular ducts, smaller in diameter than those present in the adult (Table 1) exhibited well-marked basal striation. Under the electron microscope, duct cells showed deep, complex infolding of the basal-cell membrane (Fig. 9). Between the infoldings were numerous mitochondria, and it was this arrangement that was, no doubt, responsible for the "basal striation" seen under the light microscope.

1–10 weeks.—The general appearance of the gland, as described above for the newborn rat, remained unchanged in the 1-, 2-, and 3-week stages. Staining reactions of the acinar cells and of the ducts remained constant throughout this period, as did the fine structural details of all epithelial elements. However, there was a noticeable change in size of the acini and ducts between the 3- and 4-week stages. This change was confirmed by quantitative measurements (Table 1). As a result of the increase in size of the acini and ducts, the whole gland at 4 weeks appeared compact (Fig. 2). Thereafter, between 4 and 10 weeks, no qualitative changes were apparent under the light microscope, although serous cells were seen less frequently, presumably because of the increase in extent of mucous elements. The only change noticeable under the electron microscope in this period concerned the serous cells. It was noted that zymogen granules within these cells were faintly osmiophilic in the early stages. There appeared to be a slight increase in the degree of osmiophilia during the later stages (Figs. 6 and 7), but of more interest was the relationship between these granules and endoplasmic reticulum. Particularly in glands from older animals, one frequently ob-

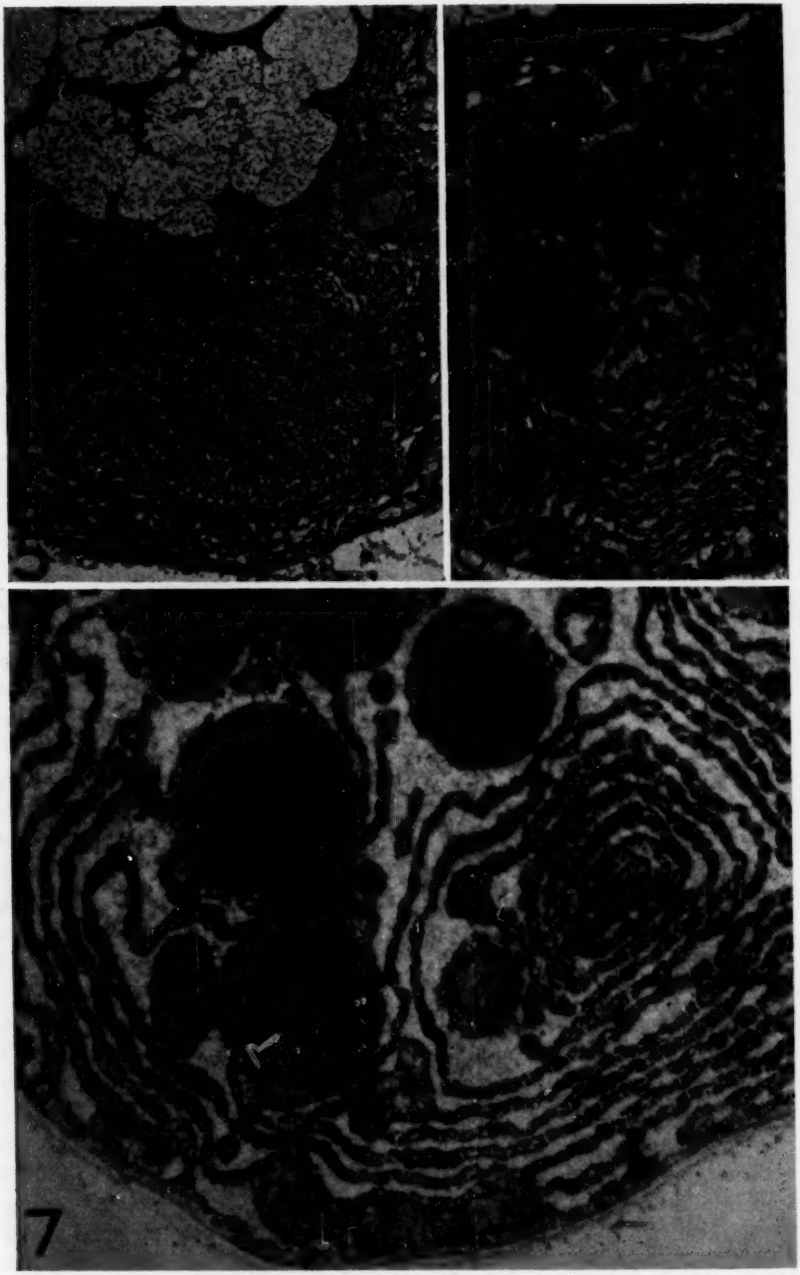

FIG. 5.—Electron micrograph of a serous cell and a portion of a mucous cell (*above*), at 1 week. The rounded nucleus of the serous cell lies centrally, and above it there is a Golgi zone, with rounded secretory granules on either side. (Mag. ×11,000.)

FIG. 6.—Electron micrograph of a portion of a serous cell at 4 weeks. Note the secretory granules and the extensive endoplasmic reticulum. A small portion of a mucous cell is present in the upper third of the micrograph. (Mag. ×12,500.)

FIG. 7.—Electron micrograph of a portion of a serous cell at 8 weeks. Note the intimate relationship between endoplasmic reticulum and secretory granules. (Mag. ×33,000.)

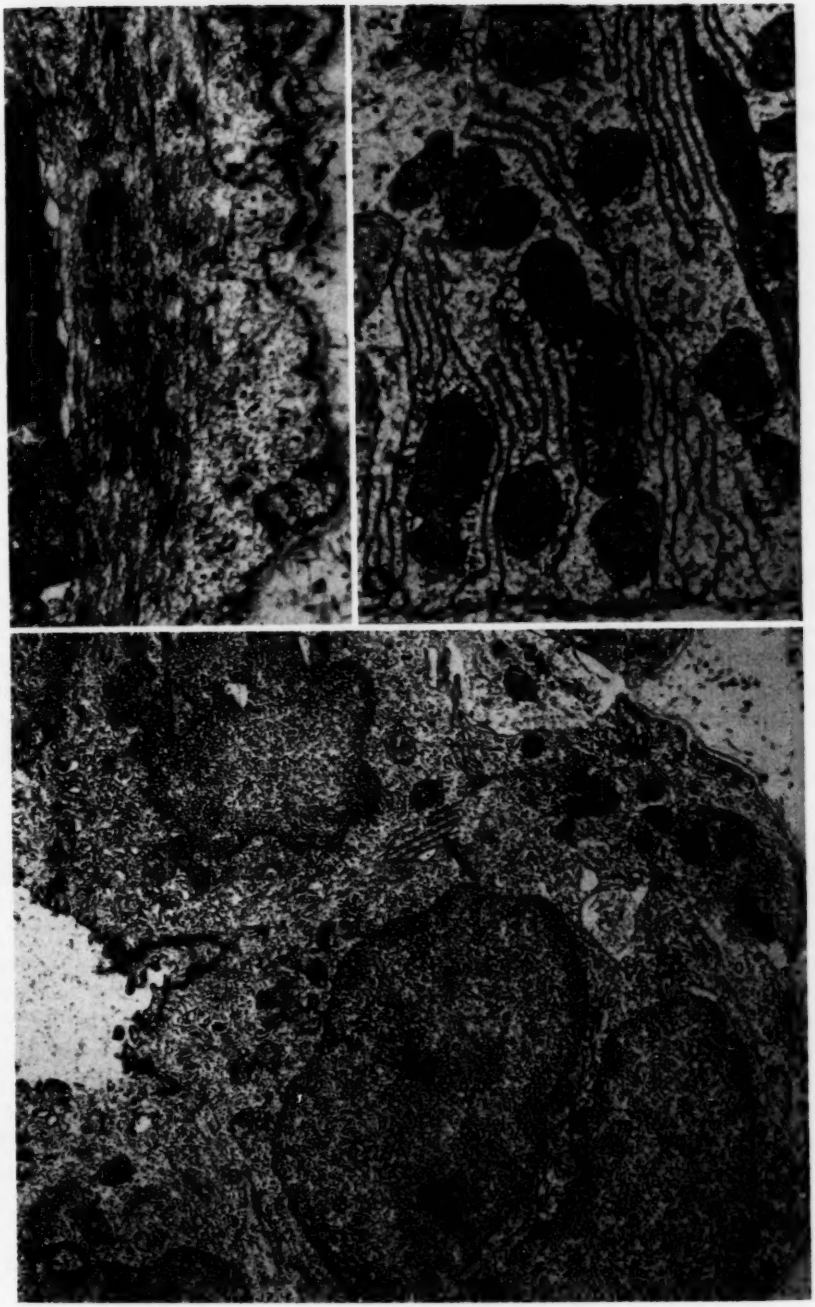

FIG. 8.—Electron micrograph of a myoepithelial cell process showing its relationship to the base of an acinar cell (*left*), to the basement membrane (*arrowed*), and the fibrillar cytoplasm. 4 weeks. (Mag. ×14,000.)

FIG. 9.—Electron micrograph of the base of a duct cell. The basement membrane is below and, above it, notice the infolding of the basal-cell membrane and numerous mitochondria. 2 weeks. (Mag. ×20,000.)

FIG. 10.—Electron micrograph of a segment of an intercalated duct, with lumen (*lower left*) and basement membrane (*upper right*). Notice the interlocking of adjoining cell membranes (*arrowed*). 2 weeks. (Mag. ×15,000.)

served granules surrounded by a granular membrane which appeared to be continuous with the double membranes (Fig. 7). Dense material, similar to that present in zymogen granules, was also seen lying between the lamellae of endoplasmic reticulum, suggesting a definite sequence of formation of secretory granules from the latter.

DISCUSSION

This study has shown that the major sublingual glands in the rat are well developed at birth. No gross histological changes occur in the postnatal period, although there is a rapid enlargement of acini and intralobular ducts in the fourth week. These findings are somewhat surprising in view of previous observations with regard to the submaxillary gland,[2] which is very immature at birth and where there is an extensive period of postnatal development and differentiation before glandular maturity is reached. In view of the adult appearance of acini, ducts, and myoepithelium in the sublingual gland at birth, it is reasonable to conclude that function is well established at this time.

During the postnatal period, the only obvious changes in the gland occur in the fourth week. At this time, there is a massive enlargement of acini and ducts (Table 1). The reason for this sudden enlargement is obscure, but it may be connected in some way with weaning, which occurs at this time in rats. With the change to a more solid diet, presumably additional mucous secretion would be required for lubrication.

Myoepithelial cells form an extensive system around acini and, to a lesser extent, around intercalated ducts in the adult. Previous work upon myoepithelium in various sites has suggested that component cells function in the evacuation of secretory products.[7, 8] However, the nature of origin of myoepithelial cells still remains in doubt. The majority of authors considers them to be epithelial in origin, although their fine structure and probable function would suggest that they arise from mesoderm. The present study can be of no assistance in answering this question, since myoepithelial cells are already well developed at birth. The problem could possibly be clarified from a study of the prenatal development of the gland.

In their study of the adult gland, Scott and Pease[5] traced the secretory product of mucous cells back to Golgi vacuoles, although they could find no similar relationship in serous cells. With regard to the latter, our own study indicates that secretory granules arise in relation to the endoplasmic reticulum. A similar relationship has also been observed previously in the acinar cells of the submaxillary gland.[9]

The important role of intralobular ducts with regard to the elaboration of the secretory product must not be overlooked.[10, 11] The presence of numerous infoldings of the basal-cell membrane, with a resultant increase in surface area of the basal regions of the duct cells, has been equated with fast transport of water and ions.[12] Such infoldings are a prominent feature of intralobular duct cells and are already well developed at birth. This, then, is further evidence in favor of the opinion that the rat sublingual gland is functional at this time.

In view of the fact that immature rats are used commonly in many aspects of dental research, it is important that findings such as these be appreciated. In the rat it is perhaps more important in the case of the submaxillary gland than of the sublingual gland, since the postnatal development of the former is more extensive and prolonged. However, one cannot correlate exactly between the degree of structural

maturity and functional activity. Although salivary glands in human infants at birth, for instance, appear structurally mature, there is some evidence to suggest physiologic immaturity.[13]

SUMMARY

The postnatal development of the major sublingual gland in rats aged from birth to 10 weeks was studied histologically and with the aid of an electron microscope. At birth, all epithelial elements within the glandular lobule were well developed. The only obvious change in the postnatal period occurred at the time of weaning (3–4 weeks), when there was a massive increase in diameter of the acini and ducts. The development of this gland was contrasted with that of the submaxillary gland in which there was a prolonged period of postnatal development and differentiation.

Additional electron-microscopic findings indicated that the secretory product of mucous acinar cells arose from Golgi vesicles, but in serous cells secretory granules appeared to arise directly in relation to endoplasmic reticulum.

REFERENCES

1. BURGEN, A. S. V. The Salivary Glands—Some Contemporary Problems, *J. Canad. D. A.*, **26**:258, 1960.
2. JACOBY, F., and LEESON, C. R. The Post-natal Development of the Rat Submaxillary Gland, *J. Anat.*, **93**:201, 1959.
3. JACOBY, F. Observations on the Post-natal Development of the Mouse Submaxillary Gland *J. Anat.*, **93**:579, 1959.
4. PLAGGE, J. C. Vital Importance of Salivary Glands to Newborn Rats, *Am. J. Physiol.*, **124**:612, 1938.
5. SCOTT, B. L., and PEASE, D. C. Electron Microscopy of the Salivary and Lacrimal Glands of the Rat, *Am. J. Anat.*, **104**:115, 1959.
6. DALTON, A. J. A Chrome-osmium Fixative for Electron Microscopy, *Anat. Rec.*, **121**:281, 1955.
7. LEESON, C. R. The Histochemical Identification of Myoepithelium,, with Particular Reference to the Harderian and Exorbital Lacrimal Glands, *Acta anat.*, **40**:87, 1960.
8. ———. The Electron Microscopy of the Myoepithelium in the Rat Exorbital Lacrimal Gland, *Anat. Rec.*, **137**:45, 1960.
9. LEESON, C. R. and JACOBY, F. An Electron Microscopic Study of the Rat Submaxillary Gland during Its Post-natal Development and in the Adult, *J. Anat.*, **93**:287, 1959.
10. BURGEN, A. S. V., and SEEMAN, P. The Role of the Salivary Duct System in the Formation of the Saliva, *Can. J. Biochem. Physiol.*, **36**:119, 1958.
11. BURGEN, A. S. V., TERROUX, K. G., and GONDER, E. The Sites of Transfer of Sodium, Potassium and Iodide in the Parotid System of the Dog, *Can. J. Biochem. Physiol.*, **37**:359, 1959.
12. PEASE, D. C. Infolded Basal Plasma Membranes Found in Epithelia Noted for Their Water Transport, *J. Biophys. Biochem. Cytol.*, **2**:203, 1956.
13. MAYER, W. B. A Comparison of the Amylase Concentration in the Saliva of Infants and Adults, *Bull. Johns Hopkins Hosp.*, **44**:246, 1959.

Some Histopathologic Changes in the Hamster as the Result of a Continuously Acting Orthodontic Appliance

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Especially during the early part of the past two hundred years in which orthodontics has been developing, there has been a constant struggle to evolve a more efficient and versatile appliance. Now there are a number of such mechanisms, all of which are efficient, for inducing orthodontic tooth movement. It was only much later that any insight into the response of the tissues to the appliance was sought. To be sure, the appliance had to be developed first, and, without it, no controlled tooth movement could have been forthcoming. It should be remembered, however, that the appliance has yet to be devised that will induce orthodontic tooth movement in a dry skull. Although some observations on the tissue response have been made, there is still considerable lag in studies of this nature. All the previous observations in this connection have been made on large animals, the expense item of which would limit the number that could be made, or on small rodents with techniques which did not produce continuous tooth movement comparable with that produced clinically. For this reason, the senior author of this article conceived an apparatus for rodents and, with the aid of several orthodontic graduate students, has conducted some experimentation, the results of which are being reported.

The histologic studies involving the monkey or the dog began with Sandstedt[1] in 1904 and were followed by Oppenheim[2] in 1911. Additional investigators who could be mentioned include Johnson, Appleton, and Rittershofer,[3] Marshall,[4] Schwarz,[5] Orban,[6] Stuteville,[7] Breitner,[8] Skillen and Reitan,[9] Lefkowitz and Waugh,[10] Frey,[11] Moyers,[12–13] and Huettener and Whitman.[14] Although various types of appliances were used and different teeth were moved in various directions, the histologic results in general were the same. Along the wall of the alveolus were found osteoblasts and new bone formation on the tension side and osteoclasts and bone resorption on the side of pressure. By demonstrating that much less relapse occurred after gingivectomies were performed, Thompson, Myers, Waterman, and Flanagan[15] recently provided evidence that the transseptal and gingival fibers outside the alveolus could also influence the orthodontic result. By showing that the same group of fibers was exceedingly

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slow in becoming rearranged, Reitan[16] has provided some histologic evidence for interpreting these results.

Sicher and Weinmann[17] should be mentioned for having shown clearly that normal molar drift in the rat is in the distal direction. By calling attention to early tissue changes incident to orthodontic tooth movement, Reitan[18–20] perhaps stimulated studies of a similar nature on the rat by groups of investigators both at Harvard[21–25] and at Illinois[26–27] dental schools. They placed a piece of rubber between the first and second molars to institute orthodontic movement. Histologic observations of the tissue revealed evidence that the normal distal drift had been altered, although the time element involved varied among these authors. Storey[28–30] used helical torsion springs to cause separation of the incisors in rabbits, rats, and guinea pigs. He also felt that the tissues surrounding the teeth were altered but differed from the observations on the rat molars in the time necessary to produce these changes. Since Storey was using a continuously growing tooth, the correlation with those observations made on the molar would not perhaps be too close.

MATERIALS AND METHODS

The first attempts at the construction of the apparatus (Figs. 1 and 2) were done with the aid of Doctors Gill and Gonzales, two orthodontic graduate students. It was sufficiently successful to warrant banding a few teeth in rats. This was done more extensively by Doctors Broussard and Bolling, two additional orthodontic students. However, the histologic slides were somewhat difficult to interpret because of the large number of roots found on the rat molar teeth. For this reason, the hamster, with its two roots on the mandibular molars, was selected for the present work.

The appliance (Figs. 1 and 2) consisted of an orthodontic band with an eyelet and two retracting coil springs. A piece of stainless-steel orthodontic band material (0.003 $\times$ 0.125 inch) was contoured and adapted to encompass both mandibular central incisors. A small eyelet was spot-welded to the lingual surface of the completed band. The retraction coil spring was constructed of 0.009-inch stainless-steel spring wire. It contained 13 turns on a 0.030-inch core. The two free ends of the spring were annealed to reduce breakage.

Approximately 60-day-old hamsters were used. Six were sacrificed for controls. The experimental animals were anesthetized by nembutal intraperitoneal injections. Approximately 6 mg. in 0.1 cc. comprised the dosage. Following anesthetization the mandibular incisors were cleaned and dried and the band cemented in place. By means of a splinter forcep, the distal end of the retraction coil spring was threaded through the interproximal space between the mandibular first and second molars. It was then tied securely to the first molar by twisting the wire tightly and placing the coiled section as closely as possible to the mesial surface of the first molar. Another coil spring was placed in the same manner on the mandibular first molar on the opposite side. The mesial free ends of the two springs were then passed through the eyelet on the lingual surface of the incisal band and tied. Each retraction coil spring was activated to produce approximately 4 ounces of applied force to the first molar tooth.* This was accomplished when the mesial ends of the springs were attached to the eyelet. Any excess wire material was trimmed away and the ends carefully turned under to prevent soft-

* As measured by a Richmond Orthodontic Stress and Tension Gauge.

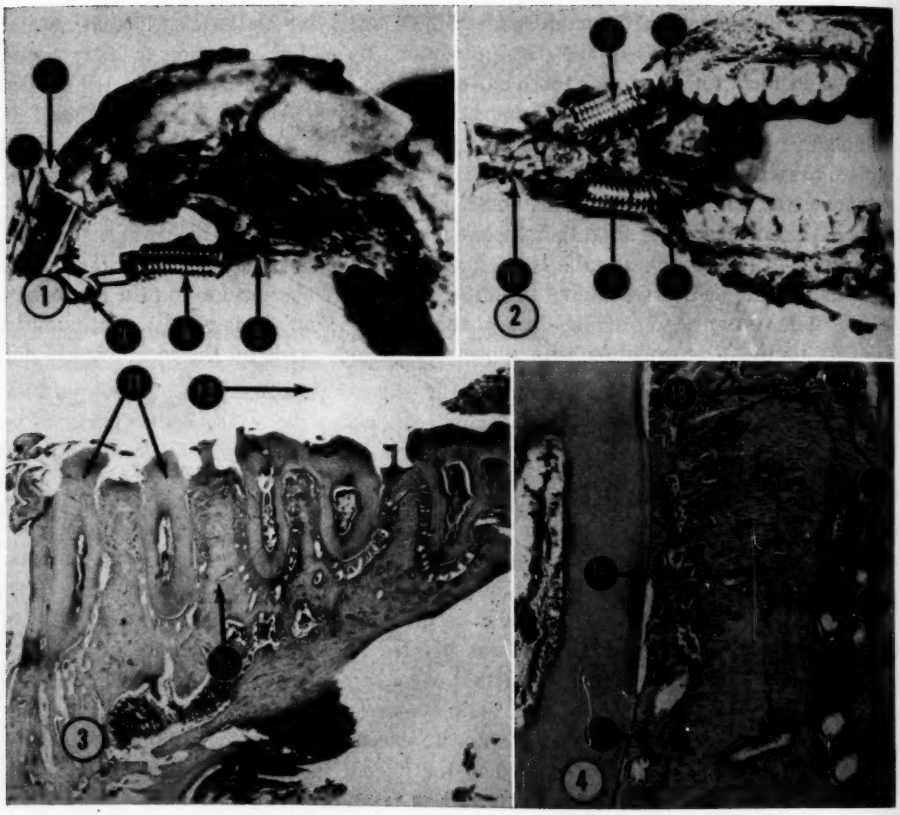

FIG. 1.—The orthodontic appliance is shown attached to the maxillary teeth. The contoured orthodontic band (*1*) is cemented to both incisor teeth (*2*). The eyelet (*3*) has been welded to the band. The coiled spring (*4*) has one end (*5*) tied around the first molar tooth and the other end tied through the eyelet.

FIG. 2.—Occlusal view of the orthodontic appliance attached to the mandibular teeth as it was used in the experiments. The two coiled springs making the appliance bilateral is shown at *6* and *7*. The wires around the first molars can be seen at *8* and *9*. The band and eyelet with the wires tied in place are shown at *10*.

FIG. 3.—Mesiodistal section through the mandibular molar region of a control hamster. The two roots of the first molar are shown at the left (*11*). In most areas the distal interradicular and interseptal alveolar bone surfaces appear smooth, indicating bone deposition, whereas the mesial surfaces appear irregular because of Howship's lacunae, thus indicating bone resorption. This indicates distal drift (*12*). Area *13* is shown in higher magnification in Fig. 4.

FIG. 4.—Higher magnification of interseptal bone area between first and second molar in area *13* in Fig. 3. The smooth interseptal bone surface with some small osteoblasts are shown at *14*. A blue-stained resting line is indicated at *15*. Rather numerous Howship's lacunae with osteoclasts are seen on the opposite bone surface (*16*). One such is indicated at *17*. The arrow at *18* indicates the direction of the distal drift.

tissue irritation. Postoperative periods consisted of 1, 2, 3, 5, 7, and 9 days with five animals in each group.

At the appropriate times the animals were sacrificed, mandibles removed, formalin-fixed, and paraffin-imbedded. Serial, 10-μ, longitudinal sections through the molar region were made. Every tenth section was mounted and stained with hematoxylin and eosin.

RESULTS

It was observed in the control hamsters, as previously noted in the rat, that normal physiologic drift of the posterior teeth was toward the distal rather than toward the mesial, as in the human (Figs. 3 and 4). Although not seen on each section in each alveolus of every molar tooth, the evidence for this drift was seen as areas of bone deposition which was manifested by the light-pink stain of osteoid and the presence of osteoblasts and as areas of bone resorption shown by the presence of Howship's lacunae and osteoclasts. The areas of bone deposition along the alveolar walls were found mesial to the mesial root of the first molar, the distal wall of the first molar inter-radicular bone, the distal wall of the interseptal bone between the first and second molars, the distal wall of the second molar interradicular bone, the distal wall of the interseptal bone between the second and third molars, and the distal wall of the third molar interradicular bone. The osteoclasts were seen on the opposite alveolar walls.

The appliance seemed to be tolerated well in all the experimental animals. Rarely was the central incisor band not retained. The only evidence of traumatic injury was sufficiently minor to be detected only microscopically. This consisted of minor epithelial sloughing distal to the first molar tooth and some inflammatory cells in the superficial gingival connective tissue (Figs. 7 and 11).

Histologic changes indicating that the appliance produced orthodontic tooth movement can be divided into two phases. The first phase (Figs. 5–8) involved the first 3 days (postoperative times 1, 2, and 3 days). The outstanding feature concerning the first molar tooth was an area of pressure necrosis in approximately the upper 20 per cent of the periodontal membrane adjacent to the alveolar crest of the interseptal bone mesial to the first root and the interradicular bone mesial to the distal root (Fig. 6.). In these two areas the periodontal membrane width was much narrowed, the collagenous fibers wrinkled, and progressive diminution of fibroblast and other nuclei. In the 3-day animals the areas were totally devoid of nuclei, thus leaving only the collagenous fibers (Fig. 6). This change was seen in all the animals sacrificed during this time. Somewhat less consistent and striking was a stretched appearance of the periodontal membrane distal to the distal root of the first molar. One would expect tension in this area to produce this result. Also in the 3-day animals, in a few instances, a small number of osteoclasts were observed on the distal surface of the interradicular bone mesial to the distal root near its apex; a similar number were observed in the apical region of the interseptal bone mesial to the mesial root of the first molar. Some indication of bone apposition and accompanying osteoblasts was evident on the mesial surface of the first molar interradicular bone. However, these changes were neither of sufficient magnitude nor present with sufficient frequency to form a definite pattern of change.

During the first 3 postoperative days the alveolar walls surrounding the second and third molar teeth showed the same evidence as that described above for the control

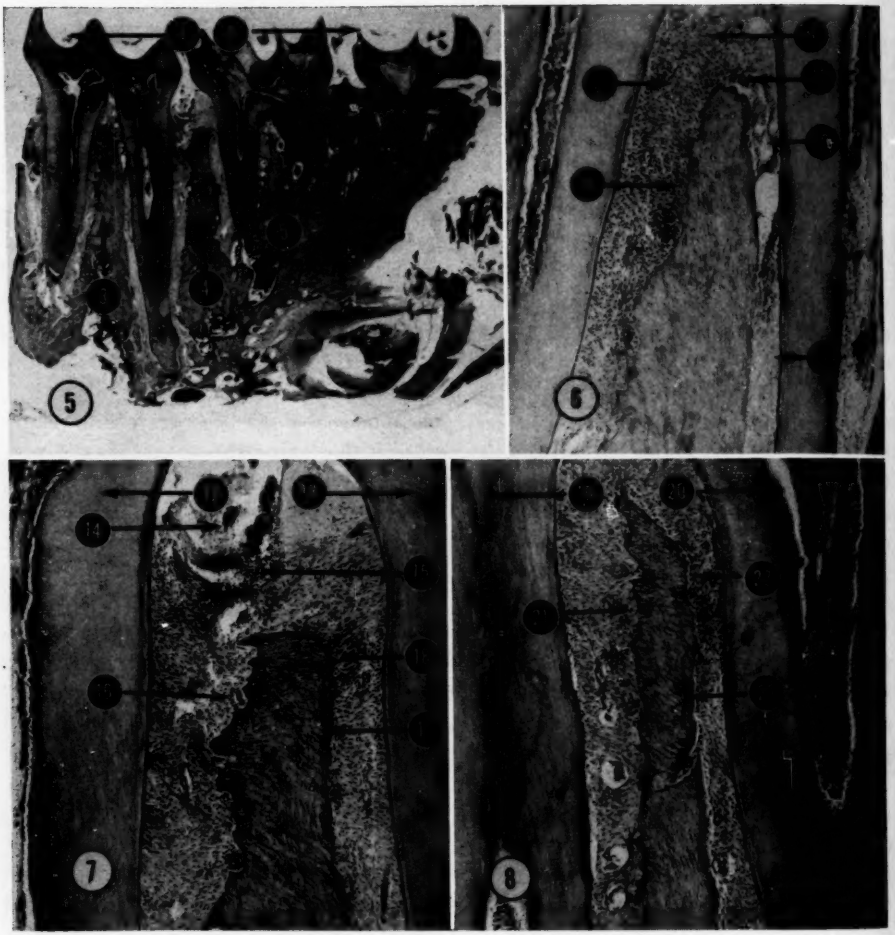

FIG. 5.—Mesiodistal section through the molar region of an animal after wearing the appliance 2 days. The first molar is shown at the left. The arrow at *1* indicates the direction of the orthodontic force on the first molar. The arrow at *2* indicates the direction of the normal distal drift. Higher magnification of areas *3*, *4*, and *5* are shown in Figs. 6–8.

FIG. 6.—Higher magnification of interradicular first molar area shown in area *3* of Fig. 5. Arrow at *6* shows direction of orthodontic force. Normal periodontal membrane with the numerous fibroblast nuclei is shown at *7*. The numerous Howship's lacunae along this mesial bone margin indicating the previous distal drift are seen at *8*. As a result of the orthodontic force, the periodontal fibers are devoid of fibroblast nuclei at *9*, and the fibers themselves seem to show some liquefaction at *10*. An osteoclast at the edge of the necrosed area is seen at *11*.

FIG. 7.—Higher magnification of interseptal areas between the first and second molars, as shown in area *4* in Fig. 5. Direction of orthodontic force is indicated by arrow at *12*. Direction of normal distal drift is indicated at *13*. Position of coiled spring wire between first and second molars is indicated at *14*. Inflammatory cells resulting from the wire are shown at *15*. Howship's lacunae containing osteoclasts along the mesial border of the bone (*16*), the blue-stained resting line (*17*), the smooth bone surface, and the small osteoblasts on the distal bone surface (*18*) all indicate that the normal distal drift was not reversed.

FIG. 8.—Higher magnification of the second molar interradicular bone area (*5*, Fig. 5) thirty sections removed from the section shown in Fig. 5. Direction of orthodontic force is shown at *19*. Direction of distal drift is indicated at *20*. Irregular bone surface indicative of resorption at *21*, resting line at *22*, and smooth bone surface with osteoblasts at *23* indicative of bone deposition indicate that distal drift was continuing.

animals, indicating that normal distal drift was still occurring (Figs. 7 and 8). Perhaps there was some diminution in the number of osteoclasts in the 3-day animals. Evidence of root resorption was found on the second molar roots in three or four instances. There was no supporting evidence to indicate that this was induced by the appliance.

The second phase comprised those animals sacrificed after 5-, 7-, and 9-day postoperative periods (Figs. 9–12). The areas of pressure necrosis mesial to the two roots of the first molar were still present (Fig. 10). However, there were also present osteoclasts in the coronal portion of the interradicular bone and at the gingival crest of the investing bone mesial to the mesial root. These were seen bordering those areas displaying the pressure necrosis, both on the gingival crest bone surface and in the periodontal membrane below the necrosed area (Fig. 10). Especially in the 9-day animal, new bone was observed somewhat consistently on the mesial surfaces of the interradicular and interseptal bone of the first molar in those sites identified as tension areas under the experimental conditions (Fig. 10).

The supporting tissues of the second and third molar teeth in the second phase manifested some features indicating that a cessation, and probably a reversal, of the normal distal drift was effectuated. This was shown especially by osteoblasts, osteoid, and blue-stained resting or reversal lines in the bone close to the osteoid (Figs. 11 and 12). This was seen on the mesial surface of the second molar interradicular bone, the interseptal bone between the second and third molars, and the interradicular bone of the third molar. The distal surfaces of these alveolar bone areas showed no osteoid and an occasional osteoclast.

It should be noted that the tissue changes described above in no instance appeared suddenly at any specific time. Rather, it was a progressive process increasing in intensity with the passage of time. For example, the pressure necrosis areas could be detected on the first day, because there was some diminution of fibroblast nuclei and some others were pyknotic. However, little significance would probably have been attached to this finding if tissues on the second and, especially, the third days had not been studied. There were some osteoclasts around these areas of necrosis in the 3-day animals, but they had become relatively prevalent at the 5-day period. Likewise, the reversal of the distal drift of the second and third molars became clearly apparent only at 5 days. Even at this time it was not seen on all the alveolar bone surfaces in all the sections.

DISCUSSION

The first statement that should be made concerning this investigation is that the appliance, when applied to the hamster, worked in a very satisfactory manner. As stated in the observations, the sole evidence of trauma resulting from the appliance was seen as a small amount of epithelial sloughing and inflammation in the superficial gingiva distal to the first molar tooth. Apparently, this was caused by the direct trauma of the wire rubbing against the tissue. The appliance was not retained in place on a few animals. But failure to maintain the appliance in the desired position was, in all cases of failure, due to unsuccessful cementation on the incisor teeth or displacement by the animal rather than failure of the ligation of the spring to the first molar tooth. Generally, the appliance was well retained and certainly merits future use in experiments with hamsters.

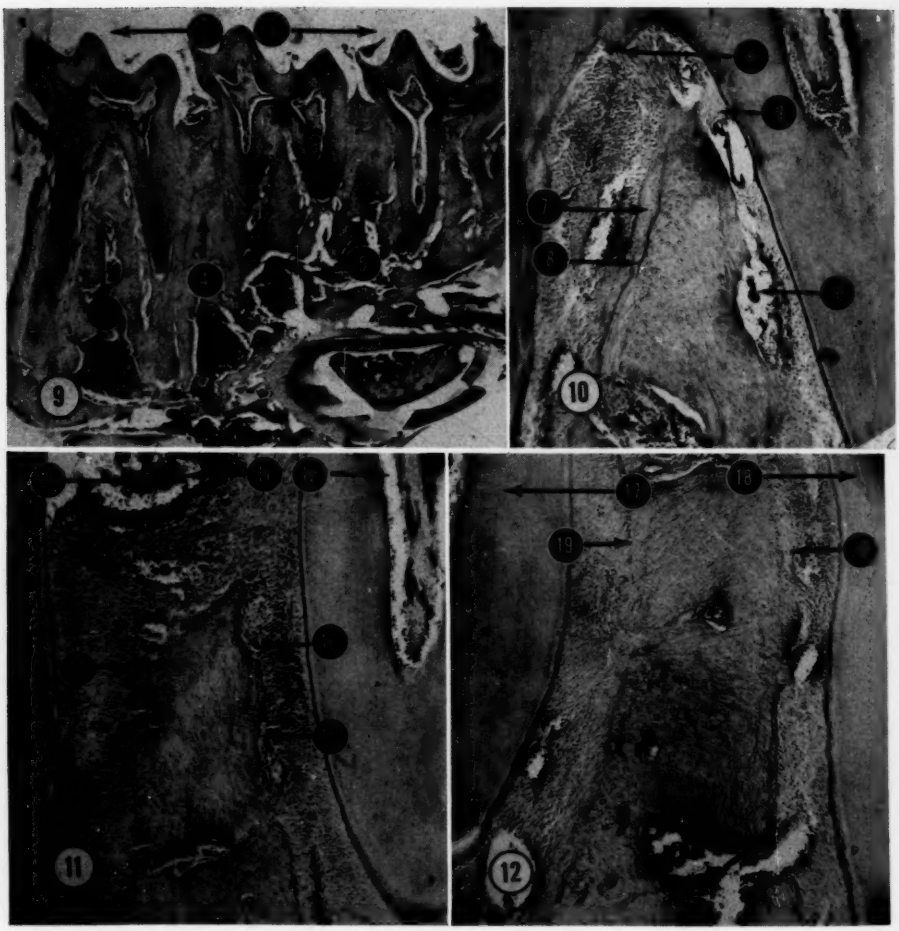

FIG. 9.—Mesiodistal section through the molar region of an animal after wearing the appliance 5 days. The first molar is at the left. The arrow at *1* indicates the direction of the orthodontic force. The arrow at *2* indicates the direction of the normal distal drift. Higher magnifications of areas *3*, *4*, and *5* are shown in Figs. 10–12.

FIG. 10.—Higher magnification of interradicular first molar area shown in area *3* in Fig. 9. Arrow at *6* indicates direction of orthodontic force. New bone deposition (*7*) and resting line (*8*) are seen on the mesial bone surface. This should be compared with the comparable area (*3*, Fig. 5, and *8*, Fig. 6) of the 2-day animal. Periodontal membrane devoid of fibroblast nuclei is shown at *9*. Osteoclasts surrounded by an area of liquefaction are seen at *10*.

FIG. 11.—Higher magnification of interseptal area between the first and second molars as shown in area *4* in Fig. 9. Arrow at *11* indicates direction of orthodontic force; *12* indicates direction of original distal drift. The debris in connection with the coiled spring wire is indicated at *13*. New bone formation on the mesial surface (*14*), the ragged distal bone edge (*15*), and osteoclasts (*16*) indicate that the mesial drift has been reversed. This should be compared with the comparable area (*4*, Fig. 5, and *16–18*, Fig. 7) of the 2-day animal.

FIG. 12.—Higher magnification of interseptal area between second and third molar as shown in area *5* in Fig. 9. Arrow at *17* indicates direction of orthodontic force; *18* indicates direction of original distal drift. New bone deposition at *19* indicates that the distal drift has been reversed. The absence of osteoclasts along the smooth bone surface at *20* indicates that distal drift reversal was not complete.

The second item that should be mentioned is the suitability of the hamster as an experimental animal for studies of this nature. It was found that the two roots of the molar teeth in the hamster were decidedly more advantageous than the five or six present in the rat in the interpretation of the direction of force and, hence, the areas in which a tissue response could be anticipated. No unusual difficulty was encountered in placing the appliance, although the hamster is a smaller animal than the rat.

In the literature review it was indicated that Sicher and Weinmann,[17] using the rat, had found that normal molar tooth movement was to the distal rather than to the mesial, as might be assumed from previous observations in the human. This fact was substantiated by a study of the tissue sections from those hamsters used as controls.

It was theorized at the beginning of the experimental work that the mesially directed force on the first molar tooth might produce a cessation or a reversal of the normal distal drift of the second and third molar teeth. Waldron[31] noted in clinical cases that, when molar teeth were moved distally, the anterior teeth also moved in the same direction. Thompson *et al.*[15] showed that gingivectomies and hence disturbance of the gingival and transseptal fibers improved retention. Reitan[16] gave histologic evidence that these fibers were very slow in becoming rearranged after rotation. Therefore, it was possible that a force on the first molar tooth could be transmitted in part to the second and third molar teeth through the transseptal and gingival connective tissue fibers. Some evidence that this was true was seen in the experimental work. The osteoclasts and new bone around the second and third molars seen for the first 3 days after the appliance was applied could have been a lag in response to the mesially directed force. It could have been, also, the fact that neither bone resorption nor new bone deposition around the first molar tooth during this early period was occurring with sufficient rapidity and magnitude to have produced enough movement of the first molar tooth to take up the slack in the transseptal and gingival fibers around the other two teeth. Which interpretation is correct is unknown at the present time. However, it can be stated that there was evidence that the distal drift was reversed. The new bone formation was found in places where it would logically be if this were true. New bone and osteoclasts were greatly diminished or absent where they would be present logically if distal drift had continued.

Good evidence was obtained that true orthodontic tooth movement did occur with the first molar tooth. This was seen during the first 3 days as an area of pressure necrosis in the upper part of the periodontal membrane close to the crest of the alveolus. This was then followed by the appearance of osteoclasts on the surface of the bone adjacent to this necrosed area. In the 5–9-day period there was also some deposition of new bone along the alveolar wall in the area of tension. This pressure necrosis, seen as an area of collagenous fibers devoid of any living cells, was mentioned by Macapanpan, Weinmann, and Brodie.[27] Whether or not this would invariably occur is unknown. It is possible that a greater and more sudden force could telescope two pathologic processes into one. In other words, the bone resorption could begin so suddenly that this necrosis period would be overshadowed and obscured. The time interval between the application of the force and the beginning of histopathologic changes is still somewhat obscure. Macapanpan[26] reported resorption within 3 hours, whereas this investigation revealed it in only small amounts until the

third day. Macapanpan found a reversal of the distal drift after 12 hours. It was not found in this study with the first molar until after 3 days and the other two molars still later. Yen and Rothblatt[24] saw resorption and new bone formation within 12 hours, and Waldo and Rothblatt,[23] stated that they observed the maximum response in 12 hours. Again, all these observations vary in the time element. This will be resolved only after different amounts of orthodontic force can be applied with the same appliance on the same species of experimental animal for specific lengths of time.

Probably the reason why most of the earlier investigators used large animals was that they were interested in duplicating the procedure as was performed clinically. As a clinician, one would be interested in getting the greatest amount of movement possible in the shortest time, compatible with least tissue trauma. Therefore, they were interested in what direction the tooth was moving and how much movement was being accomplished. Perhaps it should be pointed out that an interpretation of these factors and results can become quite complicated. One of the strongest reasons for changing to the hamster, with only the two roots to the molar teeth, was an attempt to simplify this. During the first 3 days the location of the pressure necrosis areas suggested a simple forward-tilting movement which could well be the basic direction of the force that the appliance was creating. However, when bone resorption and formation began, in the later time intervals, the tooth was actually moving beyond the normal looseness of the periodontal membrane. When this started, the tooth was no longer properly interdigitating with its antagonist, and hence the direction of the force would be changed into a complex one of retrusion, rotation, mesial bodily movement, and tilting. Probably the combination of these was somewhat different in each animal. However, the essential pattern could be seen rather clearly. In addition, however, it should be noted that these experiments were designed primarily as a study in basic pathologic processes. The primary objective in a study such as this is to determine which condition would stimulate which tissue alteration. For example, Oppenheim[2] reported the appearance of osteophites. None was seen here. Marshall[4] thought that he had soft and more elastic bone as the result of a dietary alteration. Can such a condition be produced, and will this involve a change in tissue reactions? No undermining resorption was seen in these experiments. Is this a random occurrence, or are there specific conditions under which it is produced? Osteoclasts were seen just outside the necrosed periodontal membrane area. What caused their appearance there? These are some of the questions which should be answered. The final fate of the pressure-necrosed area could not be determined in this experiment. It was still present in those animals that had worn the appliance for 9 days.

SUMMARY

An orthodontic appliance was designed to produce constant mesial movement of the mandibular first molar tooth in laboratory rodents. This appliance was found to be satisfactory for this purpose. It was found that the hamster was superior to the rat as an experimental animal in this respect.

Evidence was found that the normal distal drift of the second and third molars could be reversed by a mesial movement of the first molar. Definite evidence was seen that mesial movement of the first molar was accomplished with the orthodontic appliance.

The first pathologic process seen at the pressure site was the necrosis of the fibroblasts and other cell elements in the upper 20 per cent of the periodontal membrane. The collagenous fibers were not destroyed. The pressure necrosis was followed later by the appearance of osteoclasts adjacent to the necrosed area.

New bone formation as a result of the applied orthodontic force was seen to begin at approximately 5 days after the appliance was placed and was continued through 9 days.

The final pathologic processes involved were not determined because they extended beyond 9 days.

REFERENCES

1. Sandstedt, C. Einige Beiträge zur Theorie der Zahnregulierung, *Nordisk. Tand. Teidschr.*, No. 4, 1904; No. 1, 1905.
2. Oppenheim, A. Tissue changes, particularly of bone, incident to tooth movement, *Am. J. Orthodont.*, **3**:57, 113, 1911.
3. Johnson, A. L., Appleton, J. L., and Rittershofer, L. S. Tissue changes involved in tooth movement. *Orthodont., Oral Surg., and Rad., Intern. J.*, **12**:889, 1926.
4. Marshall, J. S., A Study of Bone and Tooth Changes Incident to Experimental Tooth Movement, *J.A.D.A.*, **17**:1221, 1930.
5. Schwarz, A. M. Tissue Changes Incidental to Orthodontic Tooth Movement, *Orthodont., Oral Surg., and Rad., Intern. J.*, **18**:331, 1932.
6. Orban, B. Biologic Problems in Orthodontia, *J.A.D.A.*, **23**:1849, 1936.
7. Stuteville, O. H. Injuries to the Teeth and Supporting Structures Caused by Various Orthodontic Appliances and Methods of Preventing These Injuries, *J.A.D.A. and D. Cosmos,* **24**:1494, Part II, 1937.
8. Breitner, C. Bone Changes Resulting from Experimental Orthodontic Treatment, *Am. J. Orthdont. and Oral Surg.*, **26**:521, 1940.
9. Skillen, W. G., and Reitan, K. Tissue Changes Following Rotation of Teeth in the Dog, *Angle Orthodont.*, **10**:140, 1940.
10. Lefkowitz, W., and Waugh, L. M. Experimental Depression of Teeth, *Am. J. Orthodont. and Oral Surg.*, **31**:21, 1945.
11. Frey, H. Tissue Changes Caused by Orthodontic Treatment, *Brit. D. J.*, **84**:52, 1948.
12. Moyers, R. E. Periodontal Membrane in Orthodontics, *J.A.D.A.*, **40**:22, 1950.
13. Moyers, R. E., and Bauer, J. L. The Periodontal Response to Various Tooth Movements, *Am. J. Orthodont.*, **36**:572, 1950.
14. Huettener, R. J., and Whitman, C. L. Tissue Changes Occurring in the Macaque Rhesus Monkey during Orthodontic Tooth Movement, *Am. J. Orthodont.*, **44**:328, 1958.
15. Thompson, H. E., Myers, H. I., Waterman, J. M., and Flanagan, Viola D. Preliminary Macroscopic Observations Concerning the Potentiality of Supra-alveolar Collagenous Fibers in Orthodontics, *Am. J. Orthodont.*, **44**:485, 1958.
16. Reitan, K. Tissue Rearrangement during Retention of Orthodontically Rotated Teeth, *Angle Orthodont.*, **29**:105, 1959.
17. Sicher, H., and Weinmann, J. P. Bone Growth and Physiologic Tooth Movement, *Am. J. Orthodont. and Oral Surg.*, **30**:109, 1944.
18. Reitan, K. The Initial Tissue Reaction Incident to Orthodontic Tooth Movement, *Acta Odont. Scandinavica,* Vol. **9,** Suppl. 6, 1951.
19. ———. Tissue Changes Following Experimental Tooth Movement as Related to the Time Factor, *D. Record,* **73**:559, 1953.
20. ———. Tissue Reaction as Related to the Age Factor, *ibid.*, **74**:271, 1954.
21. Waldo, C. M. Method for the Study of Tissue Response to Tooth Movement, *J. D. Res.*, **32**:690, 1953.
22. Rothblatt, J. M., and Waldo, C. M. Tissue Response to Tooth Movement in Normal and Abnormal Metabolic States, *J. D. Res.*, **32**:678, 1953 (abstr.).
23. Waldo, C. M., and Rothblatt, J. M. Histologic Response to Tooth Movement in the Laboratory Rat. Procedure and Preliminary Observations, *J. D. Res.*, **33**:481, 1954.
24. Yen, P. K. J., and Rothblatt, J. M. Early Tissue Changes Following Tooth Movement in the

Laboratory Rat at Varying Periods of Time after the Application of Stress, *Am. J. Orthodont.*, **41**:318, 1955 (abstr.).

25. YEN, P. K. J. Early Tissue Changes Accompanying Tooth Movement, *Harvard D. Alum. Bull.*, **15**:10, 1955.
26. MACAPANPAN, LUZ C. Early Changes Following Tooth Movement in Rats, *Am. J. Orthodont.*, **39**:956, 1953 (abstr.).
27. MACAPANPAN, LUZ C., WEINMANN, J. P., and BRODIE, A. G. Early Tissue Changes Following Tooth Movements in Rats, *Angle Orthodont.*, **24**:79, 1954.
28. STOREY, ELSDON. Bone Changes Associated with Tooth Movement. Histological Study of the Effect of Force in the Rabbit, Guinea Pig and Rat, *Austral. D. J.*, **59**:147, 1955.
29. ———. Bone Changes Associated with Tooth Movements. A Histological Study of the Effect of Force for Varying Durations in the Rabbit, Guinea Pig and Rat, *ibid.*, p. 209.
30. ———. Bone Changes Associated with Tooth Movement, *ibid.*, p. 220.
31. WALDRON, R. Reviewing the Problem of Retention, *Am. J. Orthodont.*, **28**:770, 1942.

Studies on the Non-enzymatic Release of Nitrogenous Material from Dentin

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The structure and composition of the organic part of normal dentin and enamel, as well as their alterations in caries, have been investigated (M. V. Stack, *J.A.D.A.*, **48**:297, 1954; W. C. Armstrong, *J. D. Res.*, **37**:1016, 1958). So far, the theory of initial bacterial proteolysis of keratin and collagen of the intact tooth has not been substantiated. On the other hand, there is evidence in favor of acid as the primary factor in caries initiation. It is becoming increasingly evident that the process of demineralization is accompanied by alteration of organic matter of the tooth (M. V. Stack, *J.A.D.A.*, **48**:297, 1954; K. Little, *J. Roy. Micr. Soc.*, **78**:58, 1959). The studies indicate that the interaction of acid with the organic constituents of the enamel may be a factor of considerable importance in the dynamics of early caries. This note constitutes a preliminary report on the study of the effect of acids on the solubilization of organic matter of the dentin.

Crowns of extracted intact teeth were separated from the roots at the cervical line, air-dried, and the dentinal powder was prepared from them. Twenty mg. of pooled dentinal powder were suspended in 10 ml. of bidistilled water adjusted to pH 7.0, shaken at room temperature for 15 minutes, and centrifuged. The supernatant was removed by decantation and filtered through an ultra-fine filter. The sediment was resuspended in bidistilled water to the original volume, and the procedure was repeated three times. The four supernatants thus obtained were designated S_1, S_2, S_3, and S_4. The supernatants were concentrated to about one-tenth their original volume in a desiccator, desalted by passage through a cation exchange resin Dowex-50 in H form and eluted with 0.3 N ammonia solution. The eluent was concentrated, and the desired quantity was hydrolyzed in 6.0 N HCl in sealed ampoules at 110° for 24 hours. The hydrolyzed samples free of HCl were chromatographed on Whatman No. 1 paper in n-butanol–acetic acid–water(4:1:5)v/v. The chromatograms were sprayed with ninhydrin, and isatin was employed for the detection of proline and hydroxyproline. These experiments had shown that suspension of dentin in water at a neutral pH brings about a release of a nitrogenous material which, before hydrolysis, gave one ninhydrin-positive spot close to the origin. After hydrolysis, 7 ninhydrin-positive spots were revealed. No attempt has yet been made to identify the amino acids.

Since von Kreudenstein (*N. Y. State D. J.*, **24**:343, 1958) showed by electrophoresis that dental tubuli contain water-extractable fluid components which closely correspond to those of serum proteins, experiments were performed to determine whether the nitrogenous material in the S_1 sample corresponded to the dentinal fluid. The results of these experiments have shown that the nitrogenous component of the S_1 is in no way related to serum proteins.

Supernatants S_2, S_3, and S_4 did not reveal any ninhydrin-positive spots either before or after hydrolysis. The dental powder after the fourth extraction with water at neutral pH was resuspended in a 0.02 M solution of lactate, phthalate, or citrate at pH 4.5 and shaken for an additional 48 hours. The supernatants were treated in the same way as the supernatants S_{1-4}. The results of these experiments had shown that the addition of lactate, phthalate, or citrate at pH 4.5 to the dentin after four extractions with neutral distilled water resulted in an additional release of nitrogenous material, which, upon hydrolysis, revealed a number of amino acids the fingerprints of which were different from those of the supernatant S_1. Proline and hydoxyproline were not detected.

The experiments described here have shown that dentin contains a water- and acid-soluble nitrogenous material. The origin and nature of this material is still to be determined. It is clear, however, that acid decalcification of dentin is accompanied by a loss of a certain amount of organic matter of the dentin. The possible connection between the loss of organic matter and initiation of caries is at present under study.

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A Histologic Study of the Effects of Several Periodontal Dressings on Periosteal-covered and Denuded Bone

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During the past several years there has been an increasing emphasis on alveolar-bone exposure and involvement in periodontal surgical techniques. The postoperative periodontal dressings that traditionally have been used over exposed soft tissue are now being applied to operative sites where bone is exposed. Although the reaction of soft tissue to many of the dressings used and ingredients therein have been studied by histologic methods (D. F. Mitchell, *J.A.D.A.*, **59**:954, 1959; C. M. Dixon and U. G. Rickert, *J.A.D.A.*, **20**:1458, 1933; T. D. Schaad *et al.* [abstr.], *J.A.D.A.*, **34**:11, 1956), the only reports of bone reaction have been of the effects of eugenol (Lillian Lindsay, *Fauchard's Surgeon Dentist* [London: Butterworth & Co., 1946], p. 94; E. D. Coolidge, *J.A.D.A.*, **19**:747, 1932).

Eighty Sprague-Dawley rats were divided into four groups of twenty rats each. The following periodontal surgical dressings were used as the test materials: (1) eugenol dressings: powder portion—zinc oxide, rosin powder, zinc bacitracin, liquid portion—expressed almond oil, eugenol, rosin powder; (2) non-eugenol dressing: powder portion—same as above, ointment portion—zinc oxide, hydrogenated fat; (3) a synthetic perforated dressing;* and (4) zinc oxide and eugenol.

The method used for testing was as follows: Under intraperitoneal Nembutal† anesthesia, the heads of the rats were shaved, semilunar incisions were made anterior to the ears, and flaps were retracted, exposing approximately 1 cm. of the underlying bone. In half the animals in each group (ten) the dressing to be tested was placed directly on the periosteum, while in the remaining half of the animals the periosteum was removed from the bone prior to placement of the dressing materials. The flap was then returned to place and secured with several sutures. Half the animals in each subgroup (five) were sacrificed after 7 days, and the remaining half (five) after 14 days. The heads were fixed in 10 per cent formalin and decalcified in 5 per cent formic acid. The excised tissue of the operated site was halved in a sagittal direction, imbedded in paraffin, sectioned at 6 μ, stained with hematoxylin and eosin, and examined microscopically. Ten sham-operated and five normal unoperated specimens were included for comparison.

Periodontal dressings, when placed against bone denuded of periosteum, provoked a greater inflammatory response with less evidence of repair than when placed against periosteal-covered bone. Eugenol-containing dressings elicited a greater inflammatory response with less evidence of organization than non-eugenol-containing dressings against denuded bone. The Telfa dressing caused the least inflammatory response, with more rapid repair in all instances. Sham-operated animals showed the same picture histologically as normal unoperated specimens. The site of biopsy was anterior to line of incision in areas of previously denuded bone.

While it is always dangerous to draw clinical implications from work done on animals, nevertheless the following conclusions might be applicable. A periosteal covering should be left intact when doing periodontal surgery which involves the bone. In cases where alveolar bone is denuded of its periosteum, of the dressings tried, a non-eugenol-containing dressing will provoke the least harmful response. Clinical trials which demonstrate the safety of a non-eugenol-containing periodontal dressing have been reported previously (P. N. Baer *et al.*, *Oral Surg., Oral Med., Oral Path.*, **11**:712, 1958).

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* Telfa, a Du Pont Mylar perforated film laminated to a non-woven absorbent cotton, manufactured by Bauer & Black, Chicago, Illinois.

† Nembutal®, Abbott Laboratories, North Chicago, Illinois.

Silicone Rubber as a Laboratory Duplicating Material

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The consensus of reports on physical properties of laboratory duplicating materials at the 38th General Meeting of the International Association for Dental Research held March, 1960, in Chicago was that much additional work on the accuracy of such materials was needed at the laboratory level.

The most widely used material for this purpose today is reversible hydrocolloid of the agar type. As used in the dental laboratory, it is well established that this material is subject to change due to the gain and loss of water through repeated heating and gelation cycles. It appears that this change to some degree affects its dimensional stability. Such changes in dimension are an important factor in fabricating a dental appliance, where these errors may require adjusting the resultant casting to effect the most satisfactory fit on the master cast.

The Zoller Dental Clinic Plastics Research Laboratory has been successful in its attempt to develop an improved duplicating material. Our approach to this work has been along the lines of developing a new material that will accurately duplicate the dental cast, by means of a simplified technique for

FIG. 1.—Materials and equipment required for simplified technique include vibrator, dental cast boxed in wax, tube of duplicating material, catalyst, mixing jar, and spatula.

this purpose (as shown in Fig. 1), while using to a great degree materials of proved stability and good quality. To date, tests of the new compound, consisting of certain silicones, have been carried out for twelve months on over 60 cast partial denture frameworks and many other models requiring duplication. In some instances a number of models were poured from the same duplicating mold, all with excellent results on both the laboratory and the clinical level.

Chemically, silicones are semi-inorganic polymers—organopolysiloxanes. The term "silicone" is generic. It comprises all organopolysiloxanes but may also include such materials as silcarbanes and silazanes. Organopolysiloxanes are made up of a molecular skeleton of silicon-oxygen linkages, with organic groups completing the molecule. Structurally, organopolysiloxanes may be linear or cross-linked.

The degree of cross-linking and the type of organic groups in the molecule determine, to a large extent, the physical form of the finished silicone. Dependent on structure and composition, a silicone may, therefore, have a physical form which is fluid, or it may be in resinous form, or it may even be elastomeric. The duplicating material under consideration consists of both fluid and elastomeric types.

The silicones, because of the bond energy of their silicone-to-oxygen backbone, are held together with a force one and one-half times that of a carbon-to-carbon linkage of organic materials. As a result, silicones show excellent resistance to the effects of heat, oxidation, and weathering. Their structure and composition are important when considered as a potential duplicating material, because they are water-repellent, incompatible with most organic polymers, notably indifferent to temperature variation, and possess excellent dimensional stability.

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